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JOURNAL

OF

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THE DEVELOPMENT OF BALANOGLOSSUS.

T. H. MORGAN.

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INTRODUCTION.

IN the summer of 1892 the Johns Hopkins University sent out a party of investigators to the Bahama Islands to form there a marine station.

It was my good fortune to be a member of the expedition, and I desire to express here the obligation I owe to the University for its generosity. To Prof. W. K. Brooks I am also indebted for this opportunity to join the members of the University in the marine work for the summer.

The expedition was under the immediate direction of Dr. E. A. Andrews, and to him I am grateful for numberless opportunities throughout all the time I was at the station.

The Islands of Bimini — north and south, respectively — are situated on the western edge of the Bahama Bank, and lie opposite the southern end of Florida. Owing to the absence of a regular line of boats, they are not very accessible at present. We went first to the southern end of Florida via Tampa Bay, and chartered at Key West a schooner to take ourselves and the laboratory outfit to our destination. This proved expensive, and consumed much time. A more expeditious way, and with more comfort, is by steamer to Nassau, New Providence Bahamas, from New York. Small boats frequently carry passengers from Nassau to Bimini, though at no stated times.

These details are given, since, as I shall attempt to show in a moment, Bimini proved a very exceptional locality for the marine biologist.

The Island — for usually North Bimini is meant — was selected on account of its proximity to the Gulf Stream. Between the western edge of the Bahama bank and the eastern shore of Florida, a distance of no more than 45 miles, the Gulf Stream is confined. Passing around the southern end of Florida it enters this channel and flows northward with a maximum rapidity of $5\frac{1}{4}$ miles an hour. Standing on the beach of Bimini the deep blue water of the "Stream" is seen. Here it comes to within half a mile of the bank, and in calm weather one may row or sail to the Stream in less than half an hour.

It was anticipated that the surface collecting would be excellent, and so it proved. Unfortunately, we did not have very much of the calm weather prevailing during June and July, but when a calm day arrived the fauna was both rich and varied.

There can be, I think, no question as to the suitability of the Islands for marine work, more particularly for surface-collecting. Rarely are such favorable conditions to be found for surface work on deep sea forms, where the material is within such easy reach of the shore. The relative position

of the islands themselves proved most useful for surface work. North Bimini is in the shape of a horse-shoe, while South Bimini is oval or somewhat triangular in outline and nearly fills up the opening of the horse-shoe, leaving two channels into the lagoon of North Bimini. The eastern channel is wide, but the water is shallow, while the western channel is deep and narrow, and it is this that is on the Gulf Stream side. Twice each day the lagoon fills with water through the channels between the two islands, and twice is partly emptied through the same channels. The village Alicetown is situated on the south-west corner of the horse-shoe formed by North Bimini, and therefore on the Stream side. As the tide enters the lagoon through its western channel at the maximum rate of probably more than 4 knots an hour, the surface water of the edge of the stream is drawn into the lagoon. In order to examine this water it is only necessary to push off a small boat a few yards from the shore and anchor in the midst of the current. The nets, kept stretched by the current, are drawn in at short intervals, the contents examined in glass dishes, the particular forms desired are picked out and placed in fresh water at once.

Moreover, when the tide turns, the water flowing out is found to have a different fauna from that of the incoming tide, many of the pelagic larvae having no doubt reached their destination, or served as food for the inhabitants of the lagoon; while, on the other hand, new eggs and larvae have been received from the animals of the lagoon, and these are swept by in the current bound for the Gulf Stream.

The object of my visit to the Bahamas was to study the large *Tornaria* that is found there, recorded by Weldon in 1887, and by myself in 1891. I found it to be at Bimini one of the most conspicuous features of the "tow stuff," and I was fortunate enough to get great numbers of the larvae in all stages of development, — from the quite small, free, swimming stages to the giant *Tornaria*, — and also the older and again small larva ready to transform immediately into young *Balanoglossus* as soon as supplied with sand in which to bury itself.

The larvae lived well in small aquaria, remaining at the bottom most of the time. No attempt was made to keep the young larvae for any length of time, inasmuch as an unbroken series of forms could be obtained by surface collecting where the conditions had been normal. The larvae that were caught and were in a condition to change immediately into the young *Balanoglossus*, could be kept successfully in aquaria. If they were sufficiently advanced, they went immediately into the sand supplied to them in watch crystals; if not yet ready, they developed farther at the bottom of the dish and later buried themselves in the sand. The larvae underwent the transformation with only a small percentage of abnormal forms, and no difficulty was experienced in keeping them for several weeks. The water in the aquaria was changed twice a day, and fresh sand was supplied occasionally. At a later stage in development they took into their digestive tracts large fragments of the coral sand, completely filling and distending the walls of the tube.

An attempt was made to feed the young *Balanoglossus* living in the sand on carmine to determine, if possible, the region where excretion was taking place. The coral sand was mixed up with finely powdered carmine so that each grain was partly covered with particles of carmine. The worms would enter this sand, but often came out again and wandered around the dish. A few remained for several days, and two or three for more than a week. They were then killed and afterwards examined by means of serial sections stained with picric acid. There was no evidence that the young worms absorbed any of the carmine, and the experiment gave entirely negative results. It was noticed, nevertheless, that the young worms that remained in the sand protruded in a most conspicuous way the walls of the collar-pores, so that each stuck out horn-like from the posterior edge of the collar.

Attempts made to fertilize the eggs were not successful. This was partly owing to the difficulty I had at first in getting sufficient numbers of the adult worms. An observation was made that will prove, I think, of the greatest practical advantage to any one who may have the opportunity to make a new

attempt. The presence of the adults is easily discovered by the large castings thrown out on the surface of the sand flat. These are, for the Bahama form, very large.—as thick as a man's finger. When the low tide has left the sand flats comparatively bare these coiled excrementa are conspicuous, and in most cases the posterior end of the worm itself protrudes from the surface as much, at times, as an inch. If the sand be jarred or the worm touched, the end of the body is quickly drawn out of sight, and after this any attempt made to dig the worm will not be successful. If, however, the spade is thrust rapidly into the sand before the worm has been disturbed it is easy to cut off from six inches to a foot of the hind end of the body, but impossible to get more of the worm. It is easy in this way to get large numbers of the posterior ends of the worms, but this is not very satisfactory.

Guided by a fortunate accident, and combining its data with observations obtained from the young worms kept in the aquarium, I found, but only at the end of my stay in Bimini, a method to obtain the anterior end of the worm.

It was noticed that the young worms in the aquarium made a definite tube in which they lived and which *opened at the surface of the sand*, presumably that water might be drawn down the tube. It was noticed further that if these holes were covered over they were uncovered again or new ones made. The conclusion was probably to be drawn that the adults also had holes opening to the surface. Reëxamination of the sand flats showed the truth of the deduction, and by rapidly thrusting in the spade obliquely near these holes the anterior end of the worms could often be cut off. It happened that the first attempt was made at low tide late in the afternoon, and enough worms were obtained to supply material for several days. The next attempt was made at low tide in the morning and not a single worm was obtained by repeating the process. Again and again, attempts repeated morning after morning were unsuccessful. The conclusion seemed to follow that the worms were not to be found at the top of the tubes during the morning, but that late in the afternoon they approached the surface. I left when this much seemed clear, but Dr.

Andrews, who remained longer, promised to make a new attempt when the low tide came again late in the afternoon. This was done, and Dr. Andrews tells me that they were again able to procure the worms.

A word as to the locality on Bimini where *Balanoglossus* is to be found. In the south-west corner of the lagoon, and just in front of Alicetown, is a small islet, Stokes Cay, with exposed sand flats at low tide. Here on the west side they were found in numbers. Again on the southern shore of South Bimini near its south-west corner is another locality for them. A more distant point is at the south-eastern end of North Bimini at East Wells. I did not get there, but Dr. Andrews procured *Balanoglossus* there and reports finding a large form, with red (half) bands on the body as in the Jamaica form.

Great difficulty was found in preventing the larvae from contracting when killed. One method alone gave satisfactory results. The larvae were put into an extremely dilute solution of lactic acid and left there for a few minutes until there was no response to stimuli. They were then hardened in Picrosulphuric acid or Picro-salt solution, and the usual processes followed. This method was especially successful for stages after the larvae had entered the sand. The dead and hardened worms retained the shape of the living worm almost perfectly. The lactic acid did not give favorable results with the adults, owing apparently to the large size of the worms, since maceration began at the surface before the body muscles were paralysed.

We landed on Bimini June 16, 1892, and I left July 9. Between these dates the material was collected. I am indebted to Dr. Andrews and Dr. Bigelow for preserving and sending to me additional larvae caught in the tow after my departure.

STUDY OF SURFACE VIEWS.

The series of figures given from Fig. I to Fig. II are intended to show the main features in the growth and metamorphosis of this *Tornaria*. They are all *drawn to the same scale* from the living embryos. A careful camera sketch was made in each case to give the outline and relative proportions

of the different parts. Subsequently, some details were added to the figures from the preserved animals.

The series of figures given to illustrate the development is, of course, arbitrary inasmuch as the series of changes is a continuous one, but it is convenient to be able to refer briefly to the figures as indicating definite stages of development.

The description is divided into two parts. In the first division the changes to be seen from the exterior are dealt with. The observations will be checked by information gathered from serial sections.

The second division deals more particularly with the organography and histology of the larva as gathered from surface views, dissections and serial sections.

The smallest and youngest larva obtained is shown in Fig. 1, Pl. I. It measured $1\frac{1}{4}$ mm. in length, and a little less in cross diameter. The larva is irregularly pentagonal in side view as seen in the figure. The apex of the larva, uppermost in the figure, is the anterior end. The lower (down in the figure) is the posterior end. The side where the mouth opens, to the left in the figure, is ventral and the opposite side, to the right in the figure, is dorsal (the nervous system subsequently develops on this side). Left and right are, therefore, the sides towards and away from the observer. The larva is very transparent and the tubular digestive tract lying in the middle of the body is distinctly visible from the exterior. At the anterior end are two small oval pigment spots, the so-called eyes; they are not seen in the figure.

Around the posterior end of the larva is a band of large cilia — the posterior or circular ciliated band. The posterior end of the larva is a flat plate bounded at its periphery by the circular band. This plate stretched across, like the head of a drum, is pierced near its middle by the anal opening of the digestive tract.

The anterior ciliated band, or briefly, anterior band, takes a sinuous course over the sides of the larva. Its direction may be seen by an examination of the figure. If we start with the posterior limb of the band, posterior to the mouth, we see that it runs at the side almost parallel to the circular band. About

the middle of the side it makes a sudden small upward bend, falling again before taking its upward course. Its upward or anterior course runs a little to one side of the middle line. In this part of its course it is drawn out into about eight tentacle-like projections above the surface. The band turns back (posteriorly again) to run a course almost parallel to its last. Here it is again drawn out into eight or nine tentacles. Before reaching the mid-dorsal surface it turns forward again, and is again drawn out into eight or nine tentacles.

Just beneath the apex of the larva the band turns suddenly forward to disappear in the apical plate. The structure of the apical plate is dealt with below. On the ventral lateral side of the plate a similar (or the *same*) band emerges to run downward (ventro-laterally) with its eight or nine tentacles, then turns again forward with more tentacles and again posteriorly with the eight tentacles to turn finally towards the ventral surface passing in front of the mouth-opening to repeat its course on the opposite side.

It is often desirable for purposes of description to map out the surface of the larva, and I suggest the following terms: The whole of the area included *within* the anterior band—the area containing the large black dots of the figure—may be called the circum-oral area. It is divided into four sub-regions: the ventral-lateral area, the dorsal-lateral area, the middle-lateral area, and the posterior-ventral area.

The area lying outside of the anterior band and between it and the posterior band (assuming it continuous across the apical plate) is the extra-oral. The area of the posterior plate (not seen in side view), lying within the posterior circular band, may be spoken of as the posterior plate.

Large jet-black pigment dots are found in the circum-oral area, as seen in the figure. They are seen to project in the living animal from the surface and produce the effect of large parasitic bodies clinging to the outer surface. Sections show, however, that they are constituent parts of the ectoderm as will be described later.

If we examine in detail the apex of the larva we find the ectoderm thickened, forming an apical plate containing two

small crescentic pigment spots or eyes. At the four corners of the apical plate are seen entering the four portions of the anterior ciliated band. At this stage the apical plate absorbs the ciliatic bands of the two sides, inasmuch as the bands are not after entering differentiated from the plate.

If we turn now to the internal structures, we find a relatively small digestive tract in the center of the body; it is divided into three parts,—oesophagus, stomach, and intestine. The three are separated by constrictions, and while in the larva the stomach and intestine are separated by a diaphragm-like partition, pierced in its center, this disappears in later life, and we have formed a stomach-intestine. The intestine opens by a small opening in the posterior plate; this is not in the center, but lies in the middle line nearer to the dorsal side of the larva.

A delicate thread of tissue runs from the under and inner surface of the apical plate to near the anterior end of the stomach; sections show it is part of the anterior body-cavity. In surface views this anterior body-cavity was not seen; sections show it to be a thin-walled tube that opens at the surface of the larva near the mid-dorsal line.

The next stage is shown in Fig. 2, Pl. I. This presents approximately the same surface to the observer, but somewhat more of the dorsal area is seen, and correspondingly less of the ventral side. The larva has increased in size, but the general shape is much as in the preceding stage. The anterior ciliated band follows in general the same course as before. An interesting change has taken place in the number of its tentacular processes; these have increased in each group from nine to thirteen, the additional processes having presumably been added at both ends of each group. At the twelve ends (on each side) the band shows a tendency to form new folds; and in this way, no doubt, the number of tentacles is increased. It is interesting to note that the increase in number of new tentacles is the same in each of the six (on each side) groups.

Within the circum-oral area are still found the large pigment spots.

In the interior of the larva is seen the digestive tract, with its three divisions of oesophagus, stomach, and intestine. A part of the posterior plate has been pulled in somewhat, forming what looks like a fourth division of the digestive tract; this is only accidental.

The anterior body-cavity runs from the anterior surface of the digestive tract to a point near the mid-dorsal line, where it opens to the exterior by a small pore. A delicate chord of cells connects the anterior end of this body-cavity with the under surface of the apical plate. This chord of cells is to be looked upon as a diverticulum from the body-cavity rather than a muscle-band lying outside the body-cavity.

Serial sections show that, at this stage of the development, two important organs arise. Just above the opening of the water-pore a hollow sphere of cells is found, which is the beginning of the proboscis-vesicle. Its method of origin will be described later. The other organs that appear are the posterior pair of body-cavities. This last pair—third pair of body-cavities—appears just beneath the circular band. The details of its origin are left for later treatment.

We may pass next to the *Tornaria*, when it has reached the full perfection of its larval life. Fig. 3, Pl. I, is an attempt to reproduce the larva at this stage; but it would take a more elaborate drawing to do justice to the beauty of the larva itself.

The most obvious differences between this and the preceding figure are the great increase in size ($4\frac{1}{2}$ mm. long), together with the growth in length of the tentacular processes. These stand out freely from the surface. The ciliated band runs up one side of each tentacle, crosses its top, and down the other side; thence over to the next tentacle. Each group of tentacles has approximately the same number of tentacles in it.

Another important change is the relatively smaller exposure of the circum-oral area compared with the extra-oral. It is due to the fact that the circum-oral area has sunken inwards from the general surface, so that it presents a concave face to

the exterior, while the extra-oral presents a convex surface to the observer. Coupled with this is the rolling over of the edge of the extra-oral area carrying the ciliated band, decreasing proportionately the distance between those bands that run parallel to one another. Owing to this rolling over, the bases of the tentacles lie below the general level of the extra-oral surface, as is shown to some extent in the figures. The course of the anterior band in the ventral region around the mouth is somewhat complicated, so that there is formed the arrangement that I have figured for this larva in my preceding paper (Fig. 12, Pl. XXV, *JOURNAL OF MORPHOLOGY*). For a figure of the apical plate of the larva at this stage, see in the same paper (Fig. 10, Pl. XXV).

In the apical plate the anterior ciliated band breaks into four free ends, not united across from right to left, and those on one side running parallel to one another. A surface view of the eyes of a larva of this stage is given in Fig. 19, Pl. III. (The figure of the apical plate given in my earlier paper does not properly show the eyes, owing to the fact that the pigment had been dissolved.) The structure of the eyes is treated in a later section of this paper. It will be noticed that the large pigment dots of the circum-oral area have entirely disappeared in the larger larva, although sections show that large, clear spots indicate their previous position.

The posterior ciliated band is very conspicuous, and is the chief locomotor organ of the larva. Long and large cilia project from the ring, and waves of ciliary movement may be seen in the living larva to pass over the band.

The posterior plate is still a flat ectodermal membrane stretching across the posterior end of the larva, and is pierced *near* its center by the anal opening.

The digestive tract, which is small relatively to the size of the larva, is seen within the body. The oesophagus has lengthened, and is sharply bent on itself. No other changes of importance are noticeable in the digestive tract.

The anterior body-cavity has increased enormously in size, so that it occupies a large portion of the interior of the anterior end. Its lumen extends forward almost to the apical

plate, and a blind diverticulum extends on each side posteriorly along the lateral walls of the digestive tract (not seen in figure). The opening to the exterior of this body-cavity is shown in the figure, at the small pore lying a little to the left of the mid-dorsal line.

The proboscis-vesicle has also enlarged. It now lies just above the tubular portion of the body-cavity leading to the dorsal pore. It is applied in this region to the body-cavity on its dorsal side, but is not indicated in the figure. See Fig. 32, Pl. IV. The proboscis-vesicle is, as pointed out by Met-schinkoff, contractile. I have seen it beating rythmically at this stage. A long diastole alternates with a slow systole. In what portions of its wall the contraction takes place was not determined.

The second and third pairs of body-cavities were not seen in the living animal, although sections now show that they are present.

During this period of its life the larva may be considered to have reached the climax of its pelagic existence, and it begins to undergo changes preparatory to its transformation into the young worm, having an entirely different arrangement of organs suited to new conditions of life. The change is so marked, indeed, that it might be spoken of as a metamorphosis.

The next stage drawn is shown in Fig. 4, Pl. I, but very many intermediate stages between this and the last have been studied. The outline of the larva is completely changed. Four prominent alterations may be noted. First, a decrease in size, as indicated in the relative sizes of the two drawings. Secondly, about the middle of the larva one notices a deep constriction lying at the level of the horizontal limb of the anterior ciliated band. Thirdly, the posterior plate has bulged outwards and backwards, so that instead of a flat plate it now presents a convex surface to the exterior.

In the fourth place, it will be noticed that the digestive tract, which is still connected at (or near) the center of the posterior plate, has been pulled (?) backwards during this period,

so that it now occupies a relatively different position in the interior of the larva. Instead of elongating to compensate for its protrusion posteriorly it has in fact become somewhat shorter. Due to these two changes, but principally to the former, we find, as compared with the preceding stage, a change of level of the alimentary canal. For instance, the highest point reached by the oesophagus (where it makes its bend) is considerably farther forward in Fig. 3 than in Fig. 4. The plane in which the oesophagus joins the stomach is in Fig. 3 far in advance of the level of the low limb of the anterior band, while in Fig. 4 they are in the same plane. Again, the junction of the stomach and intestine is in Fig. 3 anterior to the circular band, but in Fig. 4 far behind the same band.

If we turn now to some of the minor or less prominent changes, we find the most obvious of these to have taken place in the anterior ciliated band. The long, delicate tentacles of the earlier stages have entirely disappeared, leaving only traces behind them. The course of the anterior band, however, is still to be made out, especially in hardened specimens, and is seen as a band irregular in outline, following the same general course as in the preceding stage. An examination of stages between those of Fig. 3 and Fig. 4 shows that the tentacles shorten, become thicker, and are gradually absorbed at their bases. The details of the change will be described later.

The constriction in the middle of the larva has involved on the ventral side the ventral region of the circum-oral area, laterally the continuation of the same area, and dorsally the constriction necessarily involves the extra-oral area at the same level.

Another change will be noted involving an important region of growth, *viz.*, the extra-oral region lying posterior to the horizontal limb of the anterior band and between this and the posterior band. As compared with the preceding stage, it will be seen that this region of the body has not only relatively but absolutely increased in length antero-posteriorly. This I regard as a point of much interest, because from this

region, as we shall see, there is developed later not only the collar and its peculiar organs, but the anterior end of the body proper as well, involving the whole of the gill region.

The posterior ciliated band has grown broader, and is marked by dark, black pigment specks. The cilia are larger and thicker, and by their movements drive the larva through the water. Larvae at this stage were repeatedly caught in the surface nets, and it is from a larva just caught that the present figure was drawn. A small secondary circular band is found around the bulging posterior plate, and may be readily seen in sections and preserved larvae (it is not shown in the figure, as it was overlooked in the living animal).

In connection with the decrease in size of the body as a whole, we find the ectodermal walls considerably thickened and pigment spots scattered over the surface. The circum-oral area has decreased in surface exposure more proportionally than the extra-oral area, and its walls are correspondingly thickened. Owing to these changes the animal is less transparent than in the preceding stages. The large, brown, crescentic eyes are conspicuous at the apex of the larva.

If we examine the internal organs we find the most important change to be the shifting of the alimentary canal, noticed above. On each side of the oesophagus we find three protrusions, as shown in the figure. The anterior is the largest, the third is just forming. These six protrusions are the beginning of the gill-pouches. It may be stated that they now lie at a level far anterior to the region where they are subsequently to open to the exterior. This fact indicates that the digestive tract is to undergo farther changes before it gets into its final position.

The anterior body-cavity has enlarged, and may be dimly seen through the walls of the body. It fills up a large part of the interior of the anterior end of the larva, and opens by the dorsal pore in front of the constriction and a little to the left of the median line. Near its external opening the irregular outline of the proboscis-vesicle is seen projecting into the interior of the body-cavity at this point. A very short string of cells connects the body-cavity with the under surface of the

apical plate. Sections show that the two lateral horns of the body-cavity have grown larger.

The second and third pairs of body cavities were not observed in the living animal, but sections show their presence, and show them to have enlarged between this and the last stage.¹ The posterior pair of the two begins at the level of the posterior band, and thence extends backwards; while the anterior pair lies just in front of the posterior band, and is smaller than the last pair.

The changes that were inaugurated in the last stage continue, and the larva assumes the form shown in Fig. 5, Pl. I.

The decrease in size has continued, and more marked is the decrease in the cross-diameter, for the larva at this stage is almost as long as in the one before.

Larvae in this stage were still caught in the surface net, although the individual from which the figure was drawn had been kept for twenty-four hours in an aquarium.

The body walls have thickened, and the larva has become correspondingly more opaque. Brown pigment spots are scattered through the ectoderm, and are more conspicuous along the lines where the anterior band formerly took its course. This band is almost obliterated at this time, particularly difficult to see in the live animal, but when the walls are made opaque by the killing fluids, the remains of the band can be made out. At the apex of the larva are two conspicuous eyes. Each is crescentic in shape, with the convexities turned towards one another. The anterior end of each crescent is swollen out into a knob, as shown in the figure.

The constriction near the middle of the body and at the level of the mouth has deepened, separating an anterior egg-shaped proboscis from a posterior portion or body proper. At the ventral part of the constriction the large mouth opens directly to the exterior.

The region between the constriction and the circular band is of about the same length as before, but shows faint surface lines that correspond to the posterior border of the collar. The

¹ These were overlooked in the brief account given in an earlier paper.

region behind the circular band has increased in length and changed its general shape. It is now less conical than before and about as long as it is broad. The anus opens at the mid-posterior point of this region. The posterior band is conspicuous; it is marked by red-brown pigment and has extremely long cilia projecting from its surface. At times the cilia are actively moving and drive the body forward, at other times they lie quietly at the sides. At this age the larvae when put into an aquarium sink immediately to the bottom, and, lying in a horizontal position, are driven along by the cilia. Wave-like contractions pass along the proboscis at times, although the proboscis is not used as yet as an organ of locomotion.

The digestive tract is pulled still farther posteriorly so that the gill-pouches now lie at a level posterior to the constriction. The three divisions of the digestive tract are still found; and its walls are much thicker than in the earlier stages.

The outlines of the anterior body-cavity cannot be seen in surface view, but from sections we see that it now fills completely the interior of the anterior portion of the body, and its walls are in contact with the ectoderm. In the median dorsal line and slightly in front of the constriction, the anterior body-cavity opens to the exterior. The outline of the proboscis vesicle may be seen projecting into the body-cavity.

The outlines of the posterior body-cavities were not seen in surface views in the living animals. In preserved larvae, cleared in oil of cloves, their outlines may be distinctly seen. These two pairs nearly encircle the body, but each one of a pair is separated from its fellow in the mid-dorsal and mid-ventral lines. The anterior pair—collar cavities—or second pair of body-cavities, are much smaller than the last or third pair. This last pair extends from in front of the circular band almost to the posterior end of the body.

We come next to a stage where the larva has reached its minimum size. The individual drawn in Fig. 6, Pl. I, had been kept in an aquarium for twelve and a half hours, but it differs in no respect from other individuals of the same size that were caught in the tow-net at the surface of the Gulf

Stream. The dorsal side of the larva is turned towards the observer. Larvae of this age, kept in a dish, now went into the sand, also those newly caught buried themselves as soon as supplied with sand. Unfortunately I have not sufficient data to show definitely under what conditions the larva went into the sand. A few were seen to go into the sand when only slightly smaller than the preceding stage (Fig. 5); some of them would again come out of the sand and not go in permanently until a later stage had been reached, others seemed to remain buried. Whether the latter continued to decrease in size until they were as small as the larvae of Fig. 6 is uncertain, but from a few observations I am inclined to think that they did not continue to shrink.

The process by which the larvae went into the sand was easily seen. The tip of the proboscis was thrust obliquely or even vertically downwards between the coarse grains of sand. Its end then expanded and the body drew up nearer to the point of anchorage. The proboscis was then thrust downwards again and the process repeated. As soon as about half the proboscis was buried the whole body was lifted up, from its previous horizontal position on the sand, into a vertical position and then was rapidly drawn into the sand.

It was very noticeable that so soon as the proboscis was thrust into the sand a thick mucous was thrown out from the surface of the proboscis and collar region to which the sand granules stuck, forming an irregular tube around the animal. Often the larva disappeared in the sand in less than half a minute; at other times the body was not drawn out of sight for several minutes.

An examination of the larva shows that the most striking change, if we except the diminution in size, is the increased size of the proboscis, as compared with the rest of the body. The collar is more distinctly marked than in the preceding stage, and it will be noticed that the posterior boundary groove is not continuous across the mid-dorsal line but turns forward on each side of the mid-line, leaving a plate of ectoderm belonging to the extra-oral area between the two sides of the collar. We will return again to the description of this region. The

path of the anterior-ciliated band cannot be any longer seen in surface views. At the base of the proboscis, and a little to the left of the median-dorsal line, will be seen the external opening of the anterior body-cavity. The irregular outline of the proboscis vesicle is seen through the body-wall in this region.

Immediately behind the collar groove and quite widely separated from one another are two pits or invaginations of the ectoderm. These are the foundations of the collar pores and gill openings. At this stage they are mere pits in the ectoderm.

Dividing the body into two nearly equal parts is the circular ciliated band. Its cilia are much shorter than in the preceding stage, and the band itself where the cilia are attached is narrower. The band is marked by black-brown pigment. Scattered pigment flecks are also found over the ectoderm of the body.

The outline of the digestive tract is seen through the body-wall. It is still roughly divisible into three regions. The oesophagus shows three pairs of protrusions which are the gill-pouches. These are not fused as yet at any point with the ectoderm. The stomach is oval and its walls are, as seen in the living larva, quite thick. It is separated by a constriction from the intestine, whose walls are also proportionately thicker than before. At times, when the ventral side of the animal was turned toward the observer, the large mouth could be seen. It lies just within the collar, at the base of the proboscis.

After its entrance into the sand the larva increases at first rapidly in length, so that at the end of twelve hours it has reached the size shown in Fig. 7, Pl. I (dorsal view). I find in my notes that the larva here figured was caught in the net June 24, at 12 mid-day, and that it was drawn June 25 at three o'clock P.M. Further, that over night (or approximately for twelve hours) it had been buried in the sand. The color of the young worm when alive is milky white and the whole animal is still semi-transparent. A pair of brown-black eyes are still to be found at the anterior end of the proboscis, and the whole body is flecked with brownish pigment. The

circular band is rendered conspicuous by a narrow and somewhat irregular circle of pigment, and projecting from the band are to be found short flimmering cilia. The sand immediately around the larva is arranged into a tenacious tube; the particles held together by a mucus secreted by the animal. The young worm moves from place to place by a characteristic peristaltic movement, as in the adult.

If we examine somewhat more in detail the different regions of the body we find that the proboscis has become more elongated. At its base is found the opening of the anterior body-cavity.

The collar is much broader than in the preceding figure and more sharply separated from the body behind it. In the median dorsal line a longitudinal groove separates the two sides of the collar, and sections show that here the collar is rolling over from the sides to cover in the median piece of ectoderm that becomes the central nervous system.

The greatest change has taken place in the body behind the collar, *viz.*, in its increase in length. We have, fortunately, a well-defined and important landmark by which to gauge the growth of this region. The circular ciliated band is conspicuous on account of its dark pigment, and we are thus able to measure the relative growth of the areas before and behind the band. We find that the two regions have increased to nearly the same extent; the posterior is a little longer, but this difference was also seen in the preceding figure (Fig. 6).

In the anterior region of the body and behind the collar we find on the dorsal surface two pairs of large round openings. The first pair is the larger, and lies behind and to some extent beneath the rim of the collar; and the second pair, which is smaller, lies immediately behind the first pair. These are the external openings of the first and second gill-slits. In other words the *first and second gill-pouches of the preceding stage have opened to the exterior by four openings.*

The openings of the collar pores were not seen in the living larva, but surface views of preserved larvae and serial sections show that collar pores lie near to the first pair of gill-slits. The collar pores, we have seen, arise in point of time before

the gill-slits, and later, as we shall see, come into a secondary connection with the first pair of gill-slits.

I regard this as an important point and have given a good deal of attention to it, but we shall study it at greater length in the second part of the paper.

Spengel, in 1877, described the collar pores as arising from the first pair of gill-slits. I believe, however, that the connection is entirely a secondary one. In 1891 I saw in one larva of the New England *Tornaria* the formation of the first pair of gill-slits, and found that the ectodermal invaginations communicated with the second pair of pouches of the digestive tract. I left the question open as to whether or not this was an abnormal condition inasmuch as my results were obtained from a single larva kept for 70 hours in an aquarium. I am inclined to believe now that in this respect the development was abnormal, and that the first pair of gill pouches should have opened to the exterior before the second pair did so.

In stages of the Bahama larva, between those of Fig. 6 and Fig. 7, we find that the first pair of gill-pouches opens to the exterior some time before the second pair opens.

The outlines of the digestive tract can be seen through the body wall. The alimentary canal is a simple tube running the whole length of the body. The figure does not show its division into three parts, although the distinction is to some extent still to be found. The constriction between the stomach and intestine has almost disappeared, and it will be seen that the intestine has elongated to a very slight extent as compared with the elongation of the stomach. The 'stomach,' in fact, forms the greater length of the tube, and itself suggests the term intestine more than does the small posterior division called by that name, indicating, as before noticed, the conventionality of the terms already applied to the earlier stages.

The young worm continues to enlarge and grow in length. In Fig. 8, Pl. II, is shown a later stage (dorsal view). The larva was caught on June 23 and killed on June 27 (the exact times of day not recorded). The young worm had been in captivity for four days (or about ninety-six hours). At this

stage it had taken for the first time sand into its digestive tract. The most conspicuous change in the present form is the greater elongation of the body, while the diameter of the body has either not increased at all or only slightly so. The proboscis has enlarged and assumed more of its adult proportions. Two large pigment spots are found at the apex.

The collar has greatly altered. This is due to its elongation in the first place, and in the second to the closure of its median dorsal groove. This means that the central nervous system has sunken beneath the ectoderm of the collar, and that the collar has completely fused from side to side, obliterating all traces of its line of union. The circular groove near the posterior end of the collar is quite conspicuous, and this also is a further approach to the adult conditions. Beneath the posterior (overhanging) edge of the collar will be seen a pair of circular areas that indicate the position of the collar pores. These are seen through the walls of the collar, and their external opening lies beneath and behind the posterior edge of the collar and cannot in this position be seen at the surface. If the worm crawls directly away (down) from the observer these may often be seen as a pair of circular openings.

The body behind the collar has greatly elongated. At its posterior end it swells up and becomes more spherical. This was scarcely noticed in the preceding stage, but in all later stages it is very apparent and is not an accidental condition due to temporary contraction or expansion of the body. What the function of this knob may be I do not know.

The position of the pigment band is accurately shown and it will be seen that the growth of the body has taken place about equally in front of and behind the pigment band. It will also be noticed that the pigment band itself has become a little irregular. A slight longitudinal groove runs along the median dorsal line of the trunk and disappears beneath the collar anteriorly. This is not seen in the figure.

Behind the collar the gill region is conspicuous. Three pairs of gill-slits open at the surface ; the anterior is the largest, and the posterior is quite small as yet. Into each projects from the median and dorsal surface a bar that partly fills

up the interior of the central cavity of each slit. These bars, or tongue-bars, hang freely into the opening of the gill-slits and are formed by an ingrowth of the dorsal wall. Beneath the gill-slits the oesophagus is broad from side to side, and at its end a constriction is found. Behind this the digestive tract is a small tube, opening at the posterior end of the body. In the region of the circular band the digestive tract is swollen out and filled with a collection of sand.

On account of the greater opacity of the larva none of the changes taking place inside of the proboscis, *etc.*, could be made out.

The young worm shown in the next figure (Fig. 9, Pl. II) was caught on June 24 and the drawing made on July 1, — it had been in the aquarium about six days. The principal change is in size, but the relative proportions of the body have not materially changed. The proboscis is relatively shorter, and the collar both larger and broader.

The body behind the collar has not only elongated to nearly double its former length, but has enlarged correspondingly in other dimensions. The circular band is still marked by a circle of pigment, and is somewhat more irregular than in the preceding stage. It will be seen that the body has elongated both before and behind the band. In the present figure the part of the body behind the band is the longer, but this is due to the relative amount of contraction of the parts, for in later stages the reverse conditions were often found.

Pigment flecks cover the body and are more widely separated than in the preceding stage. Whether the pigment spots found in the ectoderm at this stage are the direct descendents of those of an earlier stage I am unable to say. The pigment spots put into the first drawings were not drawn with sufficient accuracy nor do my notes do more than point to the relative abundance in the succeeding stages. I regret this the more for they might have furnished additional data as to the method of growth.

Turning to the gill-region we find the conditions much the same as in the preceding stage. Four pairs of gill-slits open

to the exterior and each is partially divided by a tongue bar projecting into it from the dorsal wall. Each slit is relatively larger than in the preceding stage. The slits may be entirely exposed on the upper surface or may be partially over-arched by two lateral folds of the body wall. An attempt is made in the figure to show the position of these folds when the gill-slits are exposed. A constriction separates the oesophagus from the stomach-intestine. The large tube-like digestive tract runs the whole length of the body, opening at the posterior end by a large anus. In the anterior region—in front of the plane of the circular band—the walls of the digestive tract are thrown more into folds than in the region behind the band. This anterior region represents the hepatic-region of the young worm.

The posterior region shows the same bulb-like swelling of the earlier stages.

The oldest stage that is drawn *to the same scale* as the preceding stages is given in Fig. 10, Pl. II, caught June 24, killed July 5. The general enlargement of the body is obvious and the relative sizes of the organs at the anterior end have changed greatly as compared with earlier stages. The proboscis has become more pointed and is of about the same length as in the last stage. Its cross-diameter is much smaller. The collar has increased a good deal in size.

In the region of the body behind the collar the circular band of pigment may still be found, but is much more irregular than heretofore. The relative proportions of the body before and behind the band are the same as in earlier stages.

The gill-slit region covers a greater area than before and each gill-slit has increased in size. Five or six pairs of these open at the surface, while a smaller pouch that has not yet reached the surface could be seen. Only three pairs were seen in the living worm. The gill-bars are large and conspicuous.

The outline of the digestive tract may be seen through the body walls. Behind the gill-slit region the oesophagus narrows. The next portion of the tube shows an irregular series of constrictions or slight pouches. At this time this region of the

digestive tract — extending as far backwards as the circular band — has a decided yellowish-brown color that makes this region of the young worm quite conspicuous. The general color of the young worm is white, but semi-transparent, allowing the yellow of the hepatic region to be seen through the body walls. The digestive tube remains the same diameter until it reaches the bulb-like swollen end of the body, where it suddenly diminishes to an exceedingly narrow tube, flattened from side to side, connecting with the anal opening. This portion of the canal is capable of great distension to allow the passage of the large particles of calcareous sand.

The oldest worm that I obtained was drawn twelve days later than the last. Caught on June 24 and killed on July 17 — a little more than three weeks old. I am indebted for the sketch of the worm shown in Fig. ~~10~~ to Dr. E. A. Andrews. The preserved worm measured 26 mm. in length. The proportions of the body are not very different from the last stage. The circular band of pigment is still found, and the region posterior to the band is slightly the larger of the two — probably in part due to the relative amount of contraction.

Morgan
Fig. 11.

The digestive tract is indistinctly seen through the body walls as a simple tube. The tube is colored an orange yellow in the region in front of the circular band of pigment.

The number of gill-slits opening to the exterior is seven. The gill region is much more complicated than in the preceding stage. In the preserved specimen a series of closely crowded gill-slits are found, wide in front, but each succeeding gill-slit smaller posteriorly. Dr. Andrews noticed at this stage the characteristic odor of *Balanoglossus* could be made out.

BIMINI TORNARIA.

Weldon found two sorts of *Tornaria* in the Bahamas, although in his brief notice only one of these is described. I also found two sorts of *Tornaria*, but while the one described in the preceding section was very abundant, the second form was very rare. At present I have but a single individual in my possession and from this the figure was made in Pl. I, Fig. 12. I have an indistinct recollection of catching another larger

Tornaria of this sort, but it cannot be found in the series of preserved forms.

In general, the structure of the Tornaria is like the larger form shown in Fig. 3, Pl. I. Both were drawn to the same scale. The larger form I have spoken of as the Bahama Tornaria, and I take the liberty of christening the smaller form the Bimini Tornaria.

The course followed by the anterior ciliated band differs from the Bahama Tornaria in these respects: The lower horizontal limb of the anterior band does not turn forward at the middle of the side of the larva, but continues toward the dorsal surface. Before reaching the mid-dorsal line it turns back again (on each side) to follow a parallel line as far as the middle of the side of the larva. Then it turns forward along the middle lateral area. The course of the band after this follows the path characteristic for Tornaria.

The second peculiarity of the band is that it is drawn out into a few tentacle-like processes. These tentacles are neither so long nor so numerous as in the Bahama form. In this respect the Bimini form stands midway between the New England Tornaria without tentacles and the Bahama form with its large fringe of tentacles.

The third peculiarity relates to the apical plate. Two semi-circular eye-specks are found at the apex of the larva. The ciliated bands converge towards these, but the bands do not run parallel in two pairs one on each side as in the larger Tornaria, but sweep around and disappear in the region of the eyes, as in the very young larvae of the Bahama form. The apical plate of the larva was pulled in slightly and I could not determine the method of ending of the bands. When looked at from above the anterior portion of the bands are seen to approach much more nearly to one another from side to side than in the Bahama form.

The digestive tract with its three divisions is seen in the interior of the larva. On the walls of the digestive tract, where the stomach joins the intestine two oval bodies are distinctly seen on each side (on only one side of figure). These *represent the second and third pairs of body-cavities that are formed in*

contact with the endodermal walls and in the same position as in the New England Tornaria.

In this respect the Bimini form differs greatly from the Bahama Tornaria, and while it shows an approximation to the latter form in the development of tentacles, it stands nearer to the New England type in the position of its second and third pairs of body-cavities.

The anterior body-cavity is present in the larva. It is connected with the apical plate by a long process from its wall. It opens to the exterior to the left of the middle line by a single exit tube.

To what species of Balanoglossus this Tornaria gives rise I do not know.

INTERNAL (AND MICROSCOPIC) STRUCTURES.

Serial sections of larvae of Stage 1, Pl. I, show that at this early stage the body-wall is both relatively and absolutely thicker than in the later stages. The cells are not so much flattened as in the later stages. Fig. 1, Pl. III, shows a cross-section of the body-wall in the middle lateral region. At one point in the section is found a large, oval, clear spot, with a nucleus at each side; this corresponds to one of the large black dots seen on the surface of the living larva, but its pigment has been dissolved out by alcohol. The extra-oral ectoderm is at this level thinner than the circum-oral. One of the tentacles, cut in longitudinal section (in the cross-section of the body), is shown in Fig. 2. The section cuts the length of the ciliated band of the tentacle. The nuclei are found several layers in depth, and are smaller than those of the rest of the body-wall. The blastocoel cavity is continuous into the cavity of the tentacle. The cilia of the band were probably eaten off by the hardening fluid. The circular ciliated band is found to be composed of several rows of large cells fitting over one another like the shingles on a roof.

The blastocoel cavity lying between the ectodermal body-wall and the digestive tract is filled with a jelly-like fluid, and through this are found mesenchyme cells with radiating pseudopodia. A dozen such cells may be found in a single

section through the middle of the body; occasionally, one is applied to the inner surface of the body-wall, or to the outer surface of the digestive tract.

A cross-section of the oesophagus is shown in Fig. 5. It is elliptical in outline, and the internal walls (except at the sides) are richly ciliated. These ciliated cells are continuous with the ciliated bands above or below the mouth opening; and, where the oesophagus opens into the stomach, the ciliated cells are continuous with the ciliated band on the anterior wall of the stomach. *The outer wall of the oesophagus is in part covered by a layer of muscle-cells (mesenchyme).* These are found around the oesophagus at the point where the oesophagus joins the stomach, as shown in Fig. 5. The muscle-cells are elongated fibres, with the long axis around the oesophagus. Each fibre shows a strongly-refracting outer layer, and in the center lies the nucleus. *These cells are the earliest muscle-cells and only ones to differentiate from the mesenchyme.* The stomach is a large, thin-walled tube. A portion of its ventral wall, where it opens into the oesophagus, is shown in Fig. 4. The stomach is separated from the intestine by a constriction, leaving a central hole between the two cavities, richly ciliated. The walls of the intestinal division are thinner, and thrown into irregular folds. Posteriorly near (or in) the center of the posterior plate, the intestine opens by a small anus.

The anterior, first, or proboscis body-cavity is present as a simple tube; it touches the surface of the digestive tract at the anterior end of the stomach, as seen in Fig. 3 at *b.c.1*. The polygonal outlines of the cells of the stomach-wall are seen, and in the lower portion of the section the wall of the proboscis body-cavity is seen in surface view. The wall is formed by flattened cells, whose outlines are not clearly made out, the nuclei alone indicating the number of cells present. The body-cavity opens to the exterior near the mid-dorsal line, to the left, by a short (ectodermal) tube. The body-cavity sends forward a solid prolongation of its walls to join the under surface of the apical plate. Where the oesophagus joins the stomach, a pair of diverticula, hollow at first, are given off

from the body-cavity. One of these horns is shown in Fig. 4, at the side of the stomach.

A careful examination shows that at this stage no trace of the proboscis vesicle is present. Neither the second (collar) body-cavities nor the third (posterior) body-cavities are represented.

Passing to Stage 2, represented in Fig. 2, Pl. I, we gather from serial sections, both cross and longitudinal, the following: The ectodermal cells of the body wall are more flattened than in the preceding case, although the same distinction between the thickness of circum-oral and extra-oral regions is to be found as before.

A part of a longitudinal section, showing a portion of the lateral wall, is given in Fig. 6. The most conspicuous feature of such a section is the row of large cells forming the circular ciliated band. A group of three or four large cells, with flagella-like processes, form the band. Each cell has a single large cilium (or flagellum) that is split up into fibres by the hardening fluids. Above this region, the ectoderm of the extra-oral area is considerably thickened; higher up, the lower limb of the circum-oral band is cut, and above this the section passes through the circum-oral area. The posterior plate is cut in the lower portion of the figure. Its most noticeable feature is the presence of a ciliated region cut across in the section; this forms a circle of small ciliated cells on the posterior plate. It was also undoubtedly present in the preceding stage.

Surface preparation of portions of the larva verify the facts just recorded, but did not seem of sufficient importance to warrant separate figures. The nuclei of the circum-oral area are nearer together than in the extra-oral area. In the latter area, collections of nuclei are found around large, clear area, as shown in Fig. 7.

One side of the apical plate in surface view is shown in Fig. 7. On each side the anterior ciliated band is seen to end in two free ends. A pair of eyes (one in figure) are found in the apical plate. Each is situated not in the centre of the

plate, but to the ventral side, *i.e.*, nearer to one free end of the band than to the other. The structure of the eye is not easily made out. A crescent of clear cells is found, surrounded on the more central side (convexity of the crescent) by pigmented cells. Cross and longitudinal section of the larva show that all these parts of the eye lie in the surface of the ectoderm.

The structure of the digestive tract, as shown by serial sections is the same as in the preceding stage. I have added two figures for the sake of orientation. Fig. 8 cuts the digestive tract above the entrance of the tube, *tb*, leading to the true mouth, while Fig. 9 shows the external opening of this tube. The oesophagus does not open immediately to the exterior by the mouth opening, but into a depression of the surface ectoderm formed by the tube. Along this tube, *tb*, run the anterior and posterior limbs of the circum-oral band, cut in four places in the figure. The two bands on the inner side unite just below the mouth, those of the outer side unite above the mouth. These ciliated cells are directly continuous at the mouth with the ciliated cells of the oesophagus. The stomach is nearly circular in outline, somewhat flattened on the ventral side. Here the wall is thickened to form the ventral ciliated ridge of the stomach. On each side of the stomach are seen the horns of the first body-cavity, and on the dorsal side of the section the tube formed by this body-cavity is cut across. In structure the anterior body-cavity is the same as in the last stage, but larger, and the solid prolongation to the apical plate is hollowing out; the lumen is also extending further into the lateral horns.

At this stage the proboscis vesicle appears. It is important that the origin of this organ should be accurately known, and I have paid a great deal of attention to the beginning of the structure. The earliest stage that I have obtained is shown in Fig. 10. A portion of the ectodermal wall is shown in the lower part of the figure. The anterior (or proboscis) body cavity is seen at *bc'*, and the stomach wall above. Between the ectodermal body wall and the mesodermal wall of the body-cavity is found a collection of what seems to be mesenchyme cells. These are attached on one side to the ectoderm by

pseudopodial-like projections on the inner side to the wall of the body-cavity and to one another by similar processes. In every respect these cells resemble the mesenchyme cells of the blastocoel and show no trace of origin from either the mesoderm of the body-cavity on one side or from the ectoderm on the other. The cells are, I think, the beginning of the proboscis vesicle.

A much later stage in the history of the proboscis vesicle is shown in Figs. 11 and 12. The more anterior section (Fig. 11) is near the junction of the body-cavity and its short exit tube. The proboscis vesicle (*p.v.*) is more or less spherical in cross section. At one point two large cells are found running from the body wall to the proboscis vesicle, and another cell clearly inside the ectoderm closely resembles these two; whether or not this has anything to do with a proliferation of ectoderm at this spot I am unable to say. In Fig. 12 the section is nearer the periphery of the sphere. Here, one cell that forms part of the wall of the vesicle is clearly attached to the ectoderm as well.

In my previous account I have described what I believed to be a mesodermal (mesenchyme) origin of the proboscis vesicle, and I see as yet no reason to change this view. It must be admitted that it is difficult to see why so close a connection seems to be present between the ectoderm and the cells of the vesicle. On the other hand, all the direct evidence points unmistakably to a mesenchymetous origin. As before, I must take issue with Spengel on this point. It is barely possible that we are dealing with an organ having a double origin, *viz.*, from mesenchyme and ectoderm; but there is little evidence for such an assumption.

Again, at this stage, the third pair of body-cavities arises, and a little later the second-paired body-cavities arise. We may study the third pair first. Returning to Fig. 6, we find just inside of the circular ciliated band, a mass of tissue, with conspicuous nuclei and faintly stained protoplasm. This is an early stage of the last or third pair of body cavities. At this stage the tissue extends around the right and left sides, but does not meet in the mid-dorsal and mid-ventral lines. It touches

almost everywhere the inner surface of the ectoderm. A cross section of the larva cuts the body cavity along its length, and shows its close connection with the ciliated band. Such a section is figured in Fig. 13, and incidentally shows the shape of the large cells of the circular band. It is by no means an easy matter to determine with accuracy the origin of an embryonic organ, and in the present case, owing to the comparatively small number of embryos at the critical period, I have had no little difficulty in getting positive evidence on the point. Yet I feel assured that in the present case no uncertain answer is given to the problem. Longitudinal sections, cutting earlier embryos from right to left, are the only ones of value. From such a series are taken the figures shown in Figs. 14 and 15. The first series (Fig. 14, *a, b, c, d, e, f*) are from the left side, and the second (Fig. 15, *a, b, c, d, e, f, g, h*) is from the right side. The following table gives the number of the section from which each figure is taken :

Fig. 14 (left).	<i>a.</i>	13th	Fig. 15 (right).	<i>a.</i>	18th
	<i>b.</i>	31		<i>b.</i>	25
	<i>c.</i>	44		<i>c.</i>	26
	<i>d.</i>	46		<i>d.</i>	46
	<i>e.</i>	69		<i>e.</i>	60
	<i>f.</i>	82		<i>f.</i>	63
				<i>g.</i>	76
				<i>h.</i>	77

All the sections in which traces of this pair (third) of body-cavities was found, are drawn in the two series. As a rule the small collections of cells found in the larva are present in but a single section. Occasionally, as in Fig. 14, *c* and *d* (44th and 46th sections), and in Fig. 15, *g* and *h* (76th and 77th sections), the same mass is cut twice. As seen in the figure, these cells do not differ in any respect from the mesenchyme cells of the blastocoel, and when but a single cell is found in the suspected position, it cannot be affirmed that it is to become, later, part of the series ; but in most cases these cells have divided, so that an accumulation of from two, three, four or five nuclei are present, and there can then be no question of the interpretation.

It will be seen that these groups arise independently of one another, and are separated by quite wide spaces of the blastocoel cavity. Moreover, the cells are at first not in quite such close proximity to the inner wall of the ectoderm as in the later stage already studied (Fig. 6). From this early stage to the stage already studied and drawn in Fig. 6, all intermediate stages are found. *We can therefore affirm that the third pair of body-cavities arises from cells of the blastocoel space, far removed from the endoderm; that this pair of body-cavities does not have a single beginning, but comes from several groups of cells that ultimately fuse into a pair of body-cavities lying at the sides of the larva.*

It is more difficult to trace the origin of the second pair of body-cavities (collar-cavities). This is owing to the fact that there is present no landmark for finding the exact point where they appear, and also in a greater degree to the frequent collapse of the wall where they originate, rendering a determination difficult, as tangential sections are often found.

Even under these conditions the answer is definite. The body-cavities arise from mesenchyme cells, lying just beneath the ectoderm, and although I cannot state with the same positiveness as in the former case, yet there seems to be no doubt that the early beginnings appear *not to be single for each side, but to come from the fusion of several groups of cells.*

In Fig. 6, a later stage of the second body-cavity is found at *b.c.*². At this stage the body-cavity is a continuous mass on each side.

As Stage 3 represents the highest point of development reached in the pelagic life of the larva, we may study it somewhat more fully.

Small portions of the circum-oral or extra-oral area are shown in Figs. 16 and 17. In the first of these from the circum-oral area, the number of nuclei is shown, and also the presence of deeply-stained (in Haematoxylin) gland cells. In the surface view of the extra-oral area (Fig. 17), most of the nuclei are large and indistinct, but amongst these are smaller and deep-stained nuclei. Along the course of the ciliated band

the nuclei are more abundant and stain more deeply than elsewhere, but are somewhat smaller. A cross-section of one of the tentacles is shown in Fig. 18. The ciliated band, cut twice, appears on the outer sides of the tentacle. Its nuclei are more abundant than the other nuclei of the section, and the ectoderm of the band is much thicker than that over the rest of the body. The tentacle contains a central space that is merely an extension of the blastocoel space.

A view of the apical plate is shown in Fig. 19. In the center of the plate is a clear area, oval in outline, with conspicuous nuclei. The structure is best brought out by staining in Haematoxylin, but may readily be seen in unstained preparations. A small portion of a cross-section of this region is shown in Fig. 22, in the upper left corner of the figure. Here the ectodermal nuclei are seen to be less abundant, and the cells vacuolated to a very great extent. Beneath, an irregular layer of larger, more rounded nuclei separates the clear vacuolated portion from the punctated layer. To the center of this structure is attached, on the inner surface, the muscular wall of the proboscis body-cavity. I have no conception of any particular function that this organ may possess.

On each side of this body lie the two crescentic eyes, with knobbed anterior ends (Fig. 19). A dark-brown pigment is present in the eyes. The ectoderm around the eyes is also faintly marked by finely granular pigment.

Four ciliated bands end in the plate. The anterior pair run almost to the knobs of the eyes. The termination of the band is in stained specimens definitely made out; in fact the very last nucleus of the band is easily separated from the surrounding nuclei of the apical plate. The rest of the apical plate is covered by ectoderm rich in nuclei as seen both in surface views and sections. Under the apical plate the punctated layer is thicker than elsewhere more especially along the gutters between the parallel lines of the ciliated band. In the ectoderm outside of the bands clear areas are found surrounded by nuclei. Each probably corresponds to a gland. On account of the large size of the eyes I have been able to make out their structure somewhat in detail. A surface view

of the eye (*Zeiss 2, F*) is shown in Fig. 20. The pigmented area is indifferently well shown in the figure by the darker shading. The bulb at the anterior end of the eye is more richly pigmented than the rest of the eye. Towards the hollow of the crescent the eye is clearer than elsewhere. Here the ectoderm is at a much lower level than over the rest of the apical plate. This represents an invagination of ectoderm into the concavity of the crescent.

Longitudinal sections of the larva cut the eye transversely as shown in Fig. 22. The ectoderm dips down suddenly and in this region the outer ends of the ectodermal cells are clearer than those around the eye. Over the region of the pit the cuticle is somewhat separated from the surface of the ectoderm, but this is due to the action of reagents.

Fig. 21 is the outline obtained by focussing beneath the surface of Fig. 20. In the center of the bulb is a triangular space and within this another concentric line. The inner triangle encloses a space that is found, in serial sections, to communicate with the exterior at the base of the invagination on the concave side of the eye. Between the two triangles is a clear zone marked by faint lines, and outside of the larger triangle are found the clear ends of ectodermal cells, that carry the pigment in the outermost portions of the cells.

Cross-sections show us that the bulb lies deep within the ectodermal wall, that the central cavity is surrounded by cells projecting from all sides towards the center. Some of the cells of the eye are, therefore, inverted.

It seems probable that the zone between the triangles of the figure represents the thickened cuticle formed by the inner ends of the clear cells. The central cavity is not always triangular in outline but is more often irregular. Further than this I have not made out the histology of the eye. Attempts at maceration of the living specimen were not very successful.

In the early stages of the eye the anterior or bulb end was not present, the eye being a simple crescent and the invagination on the concave side being scarcely discernible. The crescent of clear cells is carried beneath the surface in the

present stage. In the later stages, as we shall see, the invagination becomes deeper and much more extensive.

To return to our study of the larva. Surface views of the posterior plate are not appreciably different from those of the extra-oral area. A portion of the posterior plate not magnified very highly (*Zciss 2, AA*) is shown in Fig. 23, Pl. IV. The periphery of the plate is bounded by the circular ciliated band (shown more in detail in a later figure). The portion of the plate drawn corresponds to the side behind the median dorsal line. Within the plate the outlines of the third pair of body-cavities (*b.c.*³) are seen; these do not meet in the middle line. Concentric with the posterior ciliated band is a much smaller band, a second circular band, and in reality the more posterior of the two. Comparing the position of this band with what we found in earlier stages we see that the distance between the two bands is greater and that the smaller band itself is narrower and more sharply defined. A figure of the band drawn to a larger scale is shown in Fig. 24. The nuclei along the course of the band are elongated and stain more deeply than those of the plate; moreover, the band is clearly defined owing to the elongation of its cells in the direction of the course of the band.

The large circular ciliated-band is shown in Fig. 25. The upper portion of the figure is part of the extra-oral area in front of the band, and that below corresponds to the posterior plate.

The band itself is composed of large cells that give rise to the large flagella-like cilia—these having fallen off in the preparation. Behind the band (below in the figure) is found a line of deep-stained nuclei that I should expect to carry cilia during life. Behind this lies the posterior plate with its few gland-cells. The region anterior to the circular band is thicker than the rest of the ectoderm (except along the bands) and contains, as seen in the figure, numerous gland-cells.

Cross-sections of the larva have in the upper half an hexagonal outline, due to the six areas between the anterior band. A portion of a cross-section, passing below the level of the mouth, is drawn in Fig. 26. The wall of the stomach is thicker than in the preceding stage and has a relatively smaller

ciliated area along its anterior wall. The anterior body-cavity (*b.c.*¹) is larger and its dorsal wall thickened. On each side of the stomach the horns of this body-cavity are cut. They have left the stomach wall at this level and pass out towards the sides of the ectodermal wall. In sections they cannot always be traced into connection with the wall, but surface views seem to show that they do unite with the wall. The entrance to the tube leading to the mouth is shown in the lower portion of this figure. Three cross-sections of the tube and of the oesophagus, cut at a higher level, are shown in Figs. 27 and 28 *a* and *b*. The first of these (Fig. 27) cuts the tube leading to the mouth at a level anterior to Fig. 26, and shows more in detail the structure of the tube. Four ciliated areas are cut, corresponding to the two limbs of the bands passing above and below the mouth at the top of the tube. In Fig. 28 *a* the section cuts the first part of the oesophagus. Two broad ciliated areas are found on opposite sides of the oesophagus, and these at the mouth are continuous with the ciliated cells of the band above and below. In Fig. 28*b* the oesophagus is cut near its union with the stomach. Its walls are thicker and the lumen ciliated. *Around the outer surface are numerous muscle cells.*

The second and third body-cavities are shown best in longitudinal sections of the larva. Fig. 29 (*Zeiss 2, AA*) is a portion of a longitudinal section of the larva along the side wall (right or left). Attached to the inner surface of the circular band is an irregular mass of cells forming the third body-cavity of that side (*b.c.*³). Above this and attached to the ectodermal wall, above the horizontal limb of the longitudinal ciliated band, is another mass of cells—the second paired cavity of that side (*b.c.*²). These body-cavities are shown more in detail in Figs. 30 and 31. Fig. 30 shows that the third pair is closely attached to the inner surface of the ectoderm. The nuclei of the mass are arranged to some extent into two distinct walls, and indications of the appearance of a lumen are found. In other larvae of this same stage the lumen of the body-cavities is quite large and the walls, therefore, well separated from one another.

The second pair of body-cavities (Fig. 31) is also attached to the ectoderm, but not so closely. The separation into two layers — somatic and splanchnic — is indicated in the preparation. Both the second and third body-cavities extend around the sides of the larva at their respective levels, but those of the right side are separated from those of the left side on the mid-dorsal and mid-ventral lines.¹

The first body-cavity, or proboscis-cavity, is seen in cross-section in Fig. 26. Its dorsal wall is found to be thickened. Surface preparations of the wall of this body-cavity show that the number of nuclei is much greater than before and each nucleus is smaller. The protoplasm of each cell has elongated to a narrow filament with the nucleus at the centre. Examined on the inner surface, the fibrous cells are seen projecting in long rows into the interior of the organ.

The relationship of the proboscis vesicle to the first body-cavity has become more intimate and more complex. The proboscis vesicle is applied quite near to the exit-tube of the left horn of the body-cavity. The proboscis vesicle is an elongated sac with thin walls; it is applied to the dorsal and dorso-posterior wall of the body-cavity. Where the surfaces are in contact anteriorly the body-cavity divides into two prolongations, one opening to the left of the median line by the ectodermal exit-tube, the other ending blindly near the surface. Looked at from the anterior end, a small portion of the surface of the proboscis vesicle is exposed between the prolongations of the body-cavity, but the greater part of the anterior wall of the proboscis vesicle is covered by the body-cavity just in front of its point of division. As looked at from the posterior end, the whole of the posterior wall of the proboscis vesicle is exposed.

A side view of the proboscis vesicle and the surrounding body-cavity is shown in Fig. 32, taken from a living larva. The left prolongation of the body-cavity, with its exit-tube, is seen. Lying against this is the small proboscis vesicle. At

¹ My statement in an earlier paper as to the absence of these body-cavities at this stage must be withdrawn. Only cross-sections were examined and the body-cavities were overlooked.

one point in the antero-ventral, where the walls of the two cavities are in contact, a proliferation is found. Such a figure does not show the right prolongation of the body-cavity that lies on the side of the proboscis vesicle towards the observer.

With this, by way of preface, we may examine a series of sections of the structures in the places indicated by the parallel lines of Fig. 32.

In Fig. 33 *A* the anterior end of the proboscis vesicle is cut ; in *B*, nearly the same conditions are found ; in *C*, we find in the upper part of the section a space, and in *D* this space opens into the blastocoel space ; in *E*, we find on each side of the proboscis vesicle the prolongation of the body-cavity, and the proboscis vesicle is exposed on its dorsal and ventral surfaces to the blastocoel space. In this same section the internal opening of the exit-tube is found on the left (right in figure) prolongation. Lastly, in *F*, the external opening of the exit-tube is seen, and this is the last section cutting through the cavity of the proboscis vesicle.

That portion of the wall of the proboscis vesicle that is exposed to the blastocoel has its cells elongated from side to side. These fibre-like cells have highly refracting borders, and the nucleus in the middle. Each cell only extends over a short portion of the wall, and has pointed ends. A portion of the wall is drawn in Fig. 34. The nuclei lie on the inner side of the cells, while the walls of the cells, represented by lines in the figure, are highly refracting. These cells have clearly differentiated into muscle-cells, and, in the living animal, during all the later stages, the proboscis-sac beats rythmically.

It has been seen that, over the area where the anterior wall of the proboscis vesicle is applied to the wall of the body-cavity, the walls of the body-cavity project into the interior of the organ itself (Fig. 32 *A*). The cavity of the blastocoel is prolonged at the anterior end of the proboscis-cavity into the space bounded by the inturned wall of the body-cavity, and this space is not filled with the same jelly-like fluid of the blastocoel, but with a more watery fluid. Fig. 32 *A* is drawn to show this condition. It is taken from a series cut longi-

tudinally, or at right angles to the last series. The plane of the section passes through the opening where the blastocoel runs into the cavity formed by the inturned wall of the body-cavity. The pavement-like cells of the wall at this point, together with the foldings of the wall, are shown. The whole structure is suggestive of an excretory arrangement, but there is no direct evidence for such a view. The section passes through the anterior end of the proboscis vesicle, and the latter is cut twice, due to its antero-ventral wall being slightly bifurcated.

In the next stage, shown in Fig. 4, Pl. I, the retrogressive changes have begun in the larval life. The general external phenomena have been described in a preceding section. From serial sections, we gather these additional facts. The ectoderm is becoming thicker, as seen in Fig. 35. This is a portion of a longitudinal section, and shows the side of the body-wall and the two body-cavities (*b.c.²* and *b.c.³*). The ectoderm between the attachment of these is shorter than in the corresponding section of the preceding stage (Fig. 29). The body-walls are thicker, and contain more nuclei arranged in several layers (as to relative position, although the cells no doubt run through the ectoderm). The increase in the circum-oral ectoderm is relatively greater, obliterating the distinction between the two layers.

The tentacles are becoming shorter. In some of the larvae that may be grouped around this "stage," the tentacles are almost entirely absorbed, as in the larva figured in Fig. 4, Pl. I; in others they are larger, so that the degree of degeneration of the tentacles is not in itself a mark of the stage reached. In fact, and this may be said of almost any single organ, the organs have rarely reached the same relative degree of change.

In Fig. 36 a cross-section of a tentacle in process of degeneration is seen. The nuclei are arranged more irregularly, and many of them seem to be disappearing. Where the tentacle is attached at its base, as seen in Fig. 40, a very irregular connection with the rest of the ectoderm is found, and in the later stages the cavity of the tentacle disappears.

The eyes on the apical plate are much more conspicuous than in the preceding stage. This is due to the greater amount of pigment developed, and to a more extensive process of invagination. A section of the apical plate is shown in Fig. 37. The plate is cut from right to left, so that both eyes appear. Between them is the central organ of the plate, but it is not so conspicuous as in the earlier stage. A deep pit leads on each side into the eye. The eyes are marked off from the surrounding ectoderm by the presence of a great amount of brown pigment, but the figure does not make the contrast between the eye and the surrounding tissue so great as in the actual sections. The outer ends of the pigment cells are clear, while the pigment is confined to an inner zone. The eyes bulge inwards, so that the punctuated layer is obliterated beneath them. The depth of the pit of the eye is greater than before, and a clear cuticle covers the outer ends of the cells bounding the pit. Sections in other planes show that the pits are continuous with the central cavity of the bulb.

The tube leading to the mouth is shorter and its walls thicker than before, but the mouth lies some distance in from the surface. The oesophagus is strongly marked with pigment. Before joining the stomach it sends out a pair of short protrusions from its antero-dorsal walls which represents the first pair of gill-pouches.

The walls of the stomach are a little thicker than in the preceding stage as seen in Fig. 38. Instead of a broad zone of ciliated cells on its ventral wall there is found now a hollow groove, and the few remaining cells with long cilia are now confined to the bottom of the groove. Whether or not all the cells of the earlier ciliated zone are turned into this groove I cannot say. The whole inner surface of the stomach seems to be covered with short cilia.

The walls of the intestine are thrown into folds but the cells are not ciliated. A bunch of cilia projects into the stomach from the periphery of the hole between stomach and intestine.

The anterior body-cavity is larger and its walls are thicker over the anterior portion of the structure. The proboscis vesicle is much as before. The second and third body-cavities

do not differ very much from the preceding stage. They are somewhat larger and the cells are in process of elongation. Fig. 39 is from a cross-section of a larva, cutting the surface of the third body-cavity, and shows the cells beginning to arrange themselves into fibres. This differentiation has gone farther in the last pair than in the collar-cavities.

The next stage represented by Fig. 5, Pl. I, shows in serial sections that the changes inaugurated in the preceding stage have gone farther. Few new changes have begun, and therefore, after a brief description, we may pass to a still later stage.

The absorption of the tenacles continues. A cross-section of the proboscis of the larva is drawn in Fig. 40. The tentacles are seen in the figure on each side of the groove as two oblong masses fused over their whole length with the extra-oral ectoderm. Each is filled with many and exceedingly small nuclei. The anterior body-cavity has not yet filled out the cavity of the proboscis. Its walls are much thicker over the anterior two thirds, and the long ridges cut in cross-section appear projecting into the lumen.

The first body-cavity is actually smaller than in the last stage, as may be seen in the surface views.

Fig. 41 is drawn from a longitudinal section of a larva at this stage (perhaps a little older). The ectoderm has thickened, and is thicker over the body than over the proboscis. The digestive tract is seen in the center of the section. The mouth opens nearly at the general surface. The thick-walled oesophagus opens into the stomach and the stomach is separated from the intestine by a double fold of the wall of the digestive tract. The section passes to one side of the opening between the two cavities.

The anterior body-cavity nearly fills up the interior of the proboscis, pushing before it the scattered mesenchyme tissue. Some of these are eventually caught between the mesodermal and ectodermal walls. Their subsequent fate I have not been able to trace with certainty. The section passes a little to one side of the proboscis vesicle.

The second and third body-cavities have distinct cavities surrounded by thin walls, the posterior pair being the larger. The cells of the walls show a differentiation into fibres arranged longitudinally along the wall, *i. e.*, parallel to the greater diameter (length) of the cavities.

A new pair of organs appears at this stage, or nearer, perhaps, to a stage between that of Fig. 5 and Fig. 6, Pl. I. These are the collar-pores that arise as a pair of invaginations of ectoderm, one on each side of the middle line just behind the edge of the collar. The earliest stage in the development of these that I have seen is shown in Fig. 42. The inturned portion of ectoderm is the anlage of the organ. The later stages we will study in older larvae.

Two of the mesenchyme cells of the blastocoel are drawn in Fig. 43. Each has a clear vacuole-like interior surrounded by a granular wall, and from the wall are sent out long pseudopodial-like processes. Often two nuclei are found in a single cell, as seen in one of the figures.

Surface views of the apical plate show that the eyes are undergoing important changes. Fig. 44 *a* is a surface view of an eye and Fig. 44 *d* is an optical section of the same. The outer line marks the limit of the pigment, but the division in the preparation is not a very sharp one. The crescentic line in Fig. 44 *a*, inside one end of the eye, marks the upper border of a hole, that leads into an invagination. In Fig. 44 *d* we have a view obtained by a deeper focus of the lens. The invaginations seen in the preceding figure leads into a rather large and very irregular cavity bounded by the outer clear ends of the eye cells. The outline of the clear zone is indicated by the middle line of the figure, and the clear zone itself marked by cross lines. The outer zone between the middle line and the outer (convex) line forms a dense border of pigment. The invagination of the eye is even more complicated, for on focusing at a deeper level the tube of the invagination sinks deeper into the bulb of the eye.

In Fig. 45 is drawn a cross-section of the eyes, taken from a longitudinal-horizontal section of the embryo. One eye (on the right) is cut at the opening of the pit. The other

eye is cut across the bulb. The lumen in Fig. 45 is bounded in the bulb by cells with clear ends, and on the outermost end of each such cell is formed a darker awn-shaped point. A round nucleus is found in the pigmented end of each such cell. Owing to the tortuous canal or invagination of the eye, it will be seen that *certain of the cells are inverted. Thus the clear cells that in an earlier stage appeared at the surface of the apical plate are now carried far within the tissue of the eye—into the bulb, and some of them are there inverted.*

Lastly, to dismiss the subject here, we have drawn in Fig. 46 the outlines of the apical plate of an older stage. At the surface a pair of holes appear, and an examination shows that the eyes have sunken farther beneath the surface ectoderm. In later stages, when the larva has entered the sand, these openings then disappear.

We may next study embryos that have reached the stage represented by Fig. 6, Pl. I. The ectoderm has become thicker, as may be seen in the cross-section shown by Fig. 48, drawn to the same scale as Fig. 41. Details in the structure of the ectoderm may be gathered from the edges of sections drawn in Figs. 49, 50 and 51.

The absorption of the tentacles has gone farther. A portion of a longitudinal section of the proboscis is drawn in Fig. 47. It represents a portion of the wall near the base of the tentacles, and the darker areas mark the last traces of the absorbed tentacles. Beneath each of the hemispherical masses is found on the inner wall a curious cell with a distinct nucleus. There is *one of these cells at each area*, but sometimes a smaller cell is attached to the large cell, as in one of the cells figured. The interior of this peculiar cell is homogeneous, and not granular. In other larvae of this same age I have found similar cells, but in no one of the other larvae were these cells so large and conspicuous as in the one figured. I do not know the fate of these cells, and they may be pathological, but this would hardly explain their constant position in connection with the tentacles. It is of course suggested from their position that they have something to do with the absorp-

tion of the degenerating tissues. That they are modified mesenchyme cells, I think, there can be no doubt ; but they are immensely larger than the mesenchyme cells, as may be seen in the figure.

The most important change that is now going on in the ectoderm is the formation of the dorsal nerve-cord, or central nervous system. Fig. 49 is taken from the posterior end of the collar region,—a little in front of the point where the collar-pores are invaginated. A central plate of ectoderm is in process of sinking beneath the surface. Its nuclei are more abundant than the nuclei of the rest of the ectoderm, and the plate as a whole stains more darkly than the rest of the ectoderm, of which it is still a part. There is in the center of the plate quite a collection of punctated substance continuous at each side by a narrow zone with the punctated layer of the ectoderm. At the sides of the plate the ectoderm is arching over.

Fig. 50, Pl. V, is the sixth section in front of the last; four more sections forward and the ectoderm is continuous with the ectoderm of the proboscis. In this figure the ectoderm has met above the nerve-chord from side to side. This represents the condition of the greater number of sections, and nowhere as yet is the chord pinched off from the ectoderm.

A second circular band presumably ciliated is found in the middle of the region between the large band and the end of the larva. This is the same band that was found encircling the posterior plate of earlier stages. A small portion of the ectoderm in the region of this band is drawn in Fig. 51. The line, generally double, of deeply stained nuclei marks the course of the band. On each side the vacuolated cells of the ectoderm, with scattered nuclei, are to be seen.

The collar-pores have reached a later stage of development at this time. The earliest stage we saw in Fig. 42 and a later stage is drawn in Fig. 52. We find in the older stages that the invagination of ectoderm is applied to the posterior end of the collar body-cavities, and that at this point the body cavity enlarges and begins to hollow out. The ectodermic tube pushes into the body-cavity and the somatic wall of mesoderm

over its end is absorbed, and then absorption of the ectodermic cells at the end of the tube also takes place, so that a communication is formed between the hollow of the body-cavity and the lumen of the collar-pores. The point of opening becomes later the funnel of the collar-pore. In Fig. 53 is drawn a portion of a cross-section at a stage when the tube of the collar-pore having pushed into the body-cavity is on the point of opening into the body-cavity by absorption of the end of the tube.

This figure contains also other points of interest. Lying ventral to the nerve chord that is in process of forming, a small pair of body-cavities are cut. These are forward extensions from the last or third pair of body-cavities. At this stage they reach as far forward as the middle of the collar region. Numerous large mesenchyme cells are found in the upper portion of the section.

Surface preparations show that the collar is distinctly marked off from the body. A zone of deeply stained nuclei is found along its posterior border.

As shown in Fig. 48, Pl. IV, the third body-cavities have filled in the space between the endodermal wall of the digestive tract and the body wall. Posteriorly each extends nearly to the end of the larva, and anteriorly we have seen each sends out a forward prolongation. The forward extensions start at the level of the collar-pores. The somatic wall of this pair of body cavities is twice as thick as the splanchnic layer, although the distinction is not seen in so small a figure. At the level of the posterior edge of the collar the third pair meet the second pair of body cavities. The second pair of body cavities was described in connection with the collar-pores. It has not opened out to the same extent as the last pair.

The first pair of body-cavities, *viz.*, the proboscis-cavity and the proboscis vesicle, almost completely fill up the blastocoel-cavity of the proboscis. A portion of a cross-section of the proboscis is shown in Fig. 54. The mesoblastic wall of the body-cavity is almost in contact with the ectodermal body-wall (the space between is due to shrinkage). Projecting from the wall into the lumen of the cavity, are longitudinal furrows.

These are hollow, and their walls are formed by the longitudinal muscle-fibres. A careful examination will show that at this stage a layer of circular muscle-fibres is formed in the outer layer of the wall. Sections tangential to the wall of the proboscis body-cavity, show most clearly this layer. In Fig. 55, taken from such a section, the two layers of muscle-fibres are indicated by the two series of parallel lines running at right angles. Those passing up and down the plate are the outer or circular muscles. The layer of circular muscles is formed from cells in the wall of the body-cavity, and not from the mesenchyme cells that still lie outside of the walls. It is interesting to note that the development of the circular layer of muscles takes place at the time when the young worm first goes into the sand.

The structure of the proboscis vesicle, and its relation to the proboscis body-cavity, has not essentially changed. Fig. 56 is taken from a cross-section of the larva, through the middle of the proboscis vesicle (*p.v.*). The ventral wall of the proboscis vesicle is turned back to some extent upon itself, leaving a square cavity with lateral wings, lying between the wall of the proboscis vesicle and the body-cavity. The space is filled with a coagulated fluid, and occasionally a mesenchyme cell is found in it. This space opens behind, directly into the blastocoel-cavity. The lateral wings leading from the central space, are filled with loose rounded cells. I have not definitely made out the origin of these cells. They may come either from the mesenchyme cells (which seems more probable) or from the walls of the body-cavity; but the cells of the latter are always on the inner side, making this view improbable. A portion of the exit-tube appears in the upper left-hand corner of the figure.

A longitudinal section through the base of the proboscis is drawn in Fig. 58. The reference-letters sufficiently show the connection between this and the last section. The postero-ventral wall of the proboscis vesicle shows differentiation into quite a thick layer of muscle cells. Numerous mesenchyme cells lie in the blastocoel spaces around the organs at this level. (*A* indicates anterior direction.)

The digestive tract is short with thick walls. The oesophagus runs immediately posteriorly from the mouth. In its dorso-lateral walls two pairs (and the beginning of a third pair) of gill-pouches are found. In Fig. 57 a side view of the oesophagus is shown. Three pairs of gill-pouches are seen projecting from the dorso-lateral walls. The first or second pairs have their median dorsal wall turned back into the cavity of the protrusion and these form subsequently the tongue bars.

The constriction separating the stomach from the intestine is disappearing. It simply pulls apart to form the lateral walls of the stomach-intestine of that region.

Between the last stage (Fig. 6) and the next stage (Fig. 7, Pl. I) a number of intermediate stages have been cut and studied. During this period two important changes have taken place, *viz.*, in the collar-pores and in the formation of the gill-slits. In other respects except an increase in size the larvae have undergone very little change.

In Fig. 59 is drawn a portion of a longitudinal (dorso-ventral) section of a stage a little older than drawn in Fig. 52. The section passes through the ectodermal opening of the invagination that gives rise to the collar-pore. The upper portion of the figure is on the collar side—the invagination lies immediately behind the collar. The bottom of the invagination is flattened out against the first gill-pouch. A double wall, the outer layer of ectoderm and the inner of endoderm, separates the cavity of the digestive tract from the lumen of the invagination. The ectoderm at the point of contact is much thinner than the ectoderm elsewhere and shows signs of degeneration. The endodermal wall is also thinner over the region of contact. The position of the funnel of the collar-pores is shown at *f*. The section does not pass through the lumen of the collar-pore which turns forward from the main invagination and which later becomes the collar-pore proper, but the position of the tube is indicated by its wall extending into the body-cavity.

A series of three sections through the region of the collar-pores at a stage older than the last are shown in Figs. 60, 61

and 62. The sections are taken from a longitudinal (dorso-ventral) series of a young worm somewhat older than Stage 6 but not so old as Stage 7. Fig. 60 is nearer to the side and Fig. 62 nearer the middle line. In Fig. 60 the opening of the collar-tube into the posterior end of the second body-cavity of one side is seen at *f*. Behind the opening a thickening of the wall is seen and this is found in the next section as well (not figured). The third section is drawn in Fig. 61 and shows that the thickening in the last is a tangential slice of the wall of the gill-pouch, and in this third section the gill-pouch opens into the ectodermal invagination that was spoken of in earlier stages as the invagination of the collar-pores. The position of the collar-pores is seen in this section by the line of cells running forward from the pit of ectoderm. Lastly in Fig. 62 is drawn the seventh section from the last. It shows the first gill-pouch with its inturned wall to form the tongue bar of later stages. By superposing the series of three sections it will be seen that it is the outer lateral portion of the pouch that protrudes towards the ectodermal invagination and unites with it to form the first gill-slit. We also see that the tongue bar is formed from a portion of the upper lateral wall of the endodermal gill-pouch.

The second gill-pouch has not yet reached the ectoderm.

We find all stages in the closure of the nerve-cord in larvae of these intermediate stages. In Fig. 63 is a cross-section, taken from the middle of the collar, and shows a later stage in the history of the cord. (The young worm had a single pair of gill-slits open.) The ectoderm by a process of sliding in from the sides has met above the median plate of ectoderm or nerve-cord, and some of the cells at the point of contact have fused in the middle line. A narrow crevice is left in the middle line above the fused ectoderm, and was seen at the surface as a longitudinal furrow. A distinct lumen is left in the upper portion of the cord. It is narrow dorso-ventrally, but wide from side to side. *This lumen may be traced through the whole length of the cord*, its proportions and size varying at different levels. The cells at the sides of the nerve-cord show a tendency to push in above the lumen of the cord, and

as we shall see, do in later stages meet above the cavity to form the dorsal wall of the tube.

Other minor points may be recorded.

The eyes are still present, but show signs of degeneration. A small central tube seems still to open at the surface.

The large circular ciliated band is disappearing. Sections show that its large ciliated cells are breaking down. Irregular pieces of these lie scattered along the former course of the band. These pieces show distinct striations resembling muscle cells. Not only are these pieces to be found along the course of the band, but they can be clearly recognized in the ectoderm some little distance away from their previous position. How it was possible for these pieces to be carried through the thick mass of ectodermal cells I do not know, but there cannot be the least doubt of the fact.

The small posterior ciliated (circular) band is also to be found at this stage. The ectodermal cells lying along the mid-dorsal line of the trunk behind the collar become smaller and stain differently from the other cells of the ectoderm.

The ectoderm of the collar is quite thick, and a distinct circular groove marks the posterior limit of this region.

Surface views of the inner side of the collar show the plate of ectoderm forming the nerve-chord. It is slightly longer than broad, as shown in Fig. 72, Pl. VI, and is continuous both in front of and behind the collar with the surface ectoderm.

Surface preparations of the proboscis show that the areas of absorption of the tentacles may still be traced. It will be found that the anterior edge of the collar coincides with the horizontal limb of the anterior circular band, and that the small dip in the middle of the band runs over the surface of the collar.

Dissections show that the oesophagus carries three pairs of gill-pouches that protrude from its upper (lateral) surface, and that the gill-pouches lie somewhat in a dorsal chamber of the oesophagus.

The constriction between stomach and intestine can still be traced.

The cells of the anterior body-cavity as shown by dissections are drawn out into exceedingly long fibres, which are arranged in longitudinal rows. Each row runs the length of the proboscis and projects far into its interior. Outside of the longitudinal fibres a plate (or layer) of circular muscles is present, but one cell in depth.

The collar-cavities are opening, but the lumen of this pair always remains filled with scattered, elongated cells.

Each of the collar-cavities sends forward a median solid protrusion almost as far forward as the proboscis vesicle.

The walls of the third body-cavity are formed of elongated cells, with the nuclei on the inner surface of the longitudinal fibre-like cells. Each body-cavity sends forward a protrusion in the middle line beneath the collar, as far forward as the beginning of the forward extension of the collar-cavities.

We may pass to a stage when the young worm has reached the stage figured in Fig. 7, Pl. I. A drawing of a longitudinal dorso-ventral section of the worm is shown in Fig. 64, drawn to the same scale as the living larva of Fig. 7. The section passes through the median dorsal line, so that the nervous system is represented by that portion of ectoderm lying beneath and detached from the collar (above). The large mouth opens to the surface at the base of the proboscis, and leads into a spacious oesophagus. The stomach intestine fills up most of the interior of the body. The walls are thick anteriorly, but quite thin behind; and in the median posterior point the anus opens to the exterior. About the middle of the body the circular band is cut, and its position is marked by pigmented cells in the ectoderm.

The body-cavities of the collar and trunk are not well shown in such a median section, but here and there portions are cut, as indicated by the darker lines in the figure.

At the base of the proboscis, and in close proximity to the dorsal wall of the oesophagus, lies the proboscis vesicle. Its ventral wall is seen to be extremely thick, due to a proliferation of cells into its interior. Touching the posterior wall of the proboscis vesicle is the median extension of the collar

body-cavity, and this extension is in close contact with the median forward extension of the third pair. Between the ventral wall of the proboscis vesicle and the dorsal wall of the oesophagus is a large blastocoel space filled with fluid. This space is continuous with the large space between the walls of the proboscis vesicle and proboscis body-cavity.

Two cross sections of the nerve-cord are shown in Fig. 65 and Fig. 66. They are from different embryos of about the same stage. In the first of these the nerve-cord has pinched off from the ectoderm above, but is still in contact with it. The large lumen of the cord is arched over by cells that have grown in from the sides of the cord. In the second figure the cord has sunken below the ectoderm of the collar, but retains a connection with the latter by a narrow line of *ectodermal* cells. The lumen of the cord is conspicuous, and the number of nuclei in cross section is gradually diminishing, as may be seen by comparison with the previous figures. On each side of the nerve-cord lie the collar body-cavities, but as yet they have not come into contact at the sides with the nervous system.

This stage is marked by the presence of two pairs of gill-slits opening at the surface of the body. Serial sections show the process of formation of the second pair of slits. The second pair of pouches protrude to meet the ectodermal ingrowth that gave rise in the early stages to the collar-pores, and subsequently served for the first gill-slit as well. The new point of contact is immediately behind the opening of the first gill-slit. The walls fuse and separate. The relative position of the openings is shown in Figs. 67 and 68, Pl. VI. The figures are portions of longitudinal (dorso-ventral) sections. The first is nearer the side of the body. The collar-pores and opening of the first gill are shown (g^1). The collar-pore is to be spoken of now as limited to the tube leading from the internal opening into the body-cavity to the external opening at the edge of the gill-slit. In Fig. 68 the opening of the second gill-slit is shown at g^2 . *It opens at the bottom of the same pit that is the common exit of the first slit and collar-pore.* The depth of this pit is without doubt subject

to changes. In fact, in the living larva the openings of the gill-slits are sometimes distinctly seen separated from one another at the surface, and at other times are partially closed in by the surrounding walls.

From the same series of sections another figure is drawn, taken nearer to the middle line; Fig. 69. Two gill-pouches are cut and a portion of a third. The two longer processes projecting into the oesophagus at t^1 and t^2 are the endodermal tongue-bars of the first and second gill-pouches. Into each of these the wall of the third body-cavity is protruding to line the interior of the tongue-bars with a layer of mesoderm. The extension of the body-cavity into the bars *between* the gill-pouches has not as yet taken place.

At this stage the dorso-anterior wall of the digestive tract begins to project forward to form the notochord. The mid-dorsal wall of the oesophagus beginning above the mouth and extending backwards beneath the collar constricts off partially from the more ventral portion of the tube, and it is the anterior end of this constriction that pushes forward as a tube into the base of the proboscis.

The third pair of body-cavities begins to fill up with fibre-like mesenchyme cells. The proliferation takes place largely, perhaps entirely, from the somatic wall. As the walls of the body-cavities come in contact with the ectoderm, obliterating the blastocoel space, the loose mesenchyme cells are caught and pressed against the walls. They are conspicuous flattened cells, and show at first no signs of degeneration. At times I have thought they seemed to migrate on the outer side through the punctated layer into the ectoderm, but I have no good evidence to support such a proposition, so improbable from *a priori* considerations. The large collection of mesenchyme cells at the base of the proboscis is very conspicuous in sections through that region.

The next stage is inaugurated by the opening at the surface of the third pair of gill-slits. They open immediately behind the second pair, and although I have not here traced all of the intermediate stages, the method seems to be the same as in the first and second gill-slits.

A figure from a horizontal section of an embryo is drawn in Fig. 70, to the same scale as the surface view of this stage. (See Fig. 8, Pl. II.) In the upper portion of the figure the collar is cut. Its interior is filled by the collar-cavity into which the collar-pores open at the sides. The interior of the body-cavity is filled with scattered muscle (or mesenchyme-like) cells not seen in the figure. Behind the collar the section passes through the upper portion of the gill-region. Portions of the tongue-bars and dividing-bars are found on each side. Into both tongue-bars and dividing or between-bars the prolongations from the third pair of body-cavities are found almost completely filling up the interior. The outer surface of the between-bars is, of course, ectodermal, but the outer exposed surface of the tongue-bars is endodermal.

The digestive tract is thrown into folds and its whole outer surface is covered closely by the splanchnic layer of the body-cavity. The walls of the digestive tract get thinner as they extend posteriorly.

From the same series we gather that the eyes have not entirely disappeared. Brown pigment, surrounding a few clear cells, mark their position.

The second and third body-cavities are filled by delicate fibre-like cells (not seen in the figure). These are most abundant at the posterior end of the third pair, when the body swells up into a bulb, and they connect the somatic and splanchnic walls.

The muscle-fibres of the walls are entirely longitudinal in both the second and third pairs of body-cavities. A double-walled septum separates these two cavities from one another.

A figure of the nervous system, taken from a larva, either just at this stage or a little prior to it, is shown in Fig. 71. It was obtained by opening the collar and removing the nervous system along with the collar. The preparation is looked at from within. The length of the nerve cord is shown by the bar of tissue running from one end of the collar to the other. For comparison there is placed alongside of this figure another, shown in Fig. 72,—a similar preparation from a larva at Stage 6. In the latter figure the bar is exceedingly short

and relatively much broader than in the first figure. It will be remembered that the nerve cord is cut off from the ectoderm in this condition.

We must conclude that the nerve cord, to keep pace with the growing collar, has elongated at the expense of its breadth.

Serial sections of the nerve cord at the stage of three gill-slits (a little later than Fig. 70), give the structure shown in Fig. 73. The cord has now sunken far beneath the surface of the ectoderm, and is connected with the latter by a band of tissue formed by the apposition of the walls of the collar body-cavities. No ectodermal cells can be found between the layers of mesoderm. The nerve cord has a relatively large lumen, but the most conspicuous feature is the scarcity of nuclei in such sections. On an average, about twelve are to be found. This fact, taken in connection with the extension of the nerve cord, as seen in surface view, leads to the conclusion that *the length is obtained at the expense of the tissue already present, this being simply pulled out.* We will return to this point again for special consideration.

Beneath the nerve cord, there is in the figure a space bounded on the outside by the collar body-cavities (*b.c.²*), but having within these walls the forward extensions of the last pair of body-cavities (*b.c.³*). In this way the cavity of the dorsal blood-vessel of the trunk is carried forward between the prolongations of the last body cavity (*b.c.³*).

In the lower part of the section are the much vacuolated cells of the dorsal wall of the digestive tract.

The forward extensions of the last pair of body-cavities, seen in the last section, reach to the anterior end of the collar where they are succeeded by a similar forward prolongation of the collar-cavities and the latter extend as far forward as the proboscis vesicle.

The notochord extends farther forward and into the region ventral to the proboscis vesicle, *i. e.*, into the region where the so-called heart was found in earlier stages. The cavity of the heart is not entirely obliterated, but is to be found above as well as anterior to the notochord. It is along the dorsal surface of the notochord that the collar-cavities reach the proboscis

vesicle and in this way the dorsal blood-vessel is continued forward.

Ventral to the notochord and at its sides, the remaining blastocoel space is filled with anastomosing mesenchyme cells.

Larvae caught on June 24 and kept till July 3, show four pairs of gill-slits opening on the dorsal surface. Horizontal sections show best the structure of the gill region. Each gill-pouch extends ventrally below the lower level of its external opening. A sort of pocket formed in the endodermal wall, but opening into the cavity of the digestive tract on one side, is found and into this hangs the tongue-bar, prolonged past the external opening ventrally into the pocket. Along a portion of the lateral walls of the pocket the endodermal walls are richly ciliated and over certain areas granular ciliated cells are found, as shown in Fig. 74.

Both in the tongue-bars and in the dividing-bars or between-bars the chitin-like skeleton of the gill-region is laid down by the endodermal cells, as shown in Fig. 74.

In each case the origin of these supporting-rods is a double one—arising from the anterior and posterior sides of their respective bars. Those in the tongue-bars are widely separated from the beginning and never unite subsequently, while the pairs formed in each between-bar are closely associated at the beginning and early unite into a single rod.

In the upper dorsal corner of the gill region the rods unite in a definite manner. The single rod in each between-bar is continuous before and behind with a rod in the tongue-bars before and behind. Each tongue-bar is, therefore, held in position at its point of origin by a rod connected with a between-bar in front and with another behind. A series of inverted double U's is found along each side of the body.

The extension of the third body-cavities into the tongue-bars is from above, *i. e.*, at their point of origin, and the cavity in the bar separates the two rods from one another.

The body-cavity found in the between-bars is a simple protrusion from the third body-cavities, continuous below with

the third body-cavities, but not above, owing to the development of the cross-rods uniting the bars.

A few other observations may be recorded for this stage. A small fifth pair of gill-slits is forming as a pouch from the upper corners of the oesophagus behind the fourth pair of slits. Its tongue-bar is forming by inturning of the dorsal wall. The cells of the stomach-intestine immediately behind the oesophagus are arranged in pouches formed by short foldings of the wall of the digestive tract. The cells in the folds are becoming more columnar, have conspicuous nuclei, and are filled with granular protoplasm. These are the cells that give the brownish color to the anterior region of the body, and are commonly spoken of as liver-cells.

The notochord is large, and fills up much of the interior of the narrow neck. In this region the mesenchyme cells that formed so conspicuous a feature in the earlier stages are no longer noticeable, and the cavity in which they were formed along each side of the neck is obliterated. They are caught between the walls, and some at least break down and disappear.

Around the posterior edge of the collar a collection of punctated substance marks the course of a nerve-tract that arises from the posterior end of the nerve cord, or rather is continuous with it.

The nerve cord is longer and also broader than in the last figure.

A blood-vessel runs around the surface of the anterior portion of the oesophagus. Dorsally it seems to be continuous with the mid-dorsal vessel, but below I could not trace its connection with the ventral vessel.

At the tip of the proboscis a few pigmented nuclei mark the former position of the larval eyes.

The reproductive organs appear at this stage, but they will be described later.

A few words may be added with respect to young worms standing in point of age between the last stage and the oldest larvae obtained. Six gill-slits have opened to the exterior. The main features of the preceding stage are to be found

here, and do not call for special notice. A portion of a cross-section through the anterior region of the collar of a worm at this stage is drawn in Fig. 75. In the upper part of the figure is seen the nervous system in cross-section. It has increased in size, and the number of its nuclei has doubled. A large lumen is present throughout the length of the chord. Beneath the nerve cord is a flat space bounded at the sides and below by the anterior median protrusion of the third body-cavities. Beneath this lies the flattened notochord, cut just in front of its opening into the oesophagus. It has a large lumen, wide from side to side. Ventral to the notochord are two chitin-like rods that are formed on each side in the folds of entoderm. These are the supporting-rods of the neck, united anteriorly into a single rod in the neck and base of the proboscis. A large space lying between the rods in the figure is probably an artefact. The splanchnic layer of the mesoderm on each side is differentiated along the rods into a thick muscle-layer. Anterior to this region a pair of forward lateral extensions of the collar body-cavity follows the course of the rod, and forms a layer of muscles, as is shown at *b.c.2*, in Fig. 77.

It is very difficult to follow the course of the blood-vessels in the young worms, owing to the elasticity of the walls; so that at times a large vessel may be found in section distended with blood, and at other times the same vessel cannot be found, owing to the absence of blood, and the collapse of the walls. In one worm of this stage a very large accumulation of blood was found in the blood-vessel between the notochord and proboscis vesicle, so that the blood-vessel seemed suspended from the dorsal well of the notochord into the cavity of the proboscis vesicle. From the blood-vessel the blood is continuous with the irregular spaces between the inturned cells of the proboscis-cavity. At the base of the proboscis vesicle the vessel is nearly obliterated, and inasmuch as the dorsal vessel a few sections back is empty of blood, no connection between the two could be seen. A median dorsal and a median ventral blood-vessel can always be found in the body proper, formed by the apposition of the walls of the second and third pairs of body-cavities.

A pair of reproductive gonads is present at this stage behind the last gill-slit, but a description of these will be deferred till a later stage.

We may now pass to a description of the oldest worms of the series that had been kept from June 24 to July 17, drawn in Fig. 11, Pl. II. It is unnecessary to describe completely the structure of the specimens, as a great deal of repetition would be necessary. The points calling for special attention are as follows :

Seven pairs of gill-slits open at the surface behind the collar ; the anterior ones are very large compared with the preceding stages. The external openings are not so extensive as the gill-pouches themselves ; these are prolonged both above and below the openings, as explained in the preceding section. The rods are more largely developed than before. A cross-section of a tongue-bar and its posterior between-bar is given in Fig. 76, taken from a horizontal series. The rods are shaded darker, and it will be seen that the rods in the tongue-bar are widely separated, while those in the between-bar are fused together except at the innermost end, where they show a double origin.

The body-cavity in the tongue-bars fills nearly the whole of the interior of the bar. A small cavity is seen on the inner side of the bar surrounded at the sides by the endoderm, and on the outer side by the wall of the body-cavity. I believe this to represent a blood-vessel, although I have no definite proof of it. In the between-bar the body-cavity is confined at this level (where it opens to the exterior) to the outer region of the bar, where the bar is covered by ectoderm. A small triangular space is found towards the inner side of the body-cavity, bounded by endoderm and mesoderm. The space contains a coagulated material resembling blood, and the space seems to be a blood-vessel. At times, a space is found between the knobbed ends of the chitin-rods, but I cannot say that this is a blood-vessel. Both tongue-bars and between-bars have long cilia over the adjacent sides of the bars.

A cross-section of the neck or narrow base of the proboscis is shown in Fig. 77. On the dorsal surface, and laterally, the ectoderm is much thickened with a large amount of punctated substance. The ectoderm of this region is directly continuous in the median dorsal line with the nerve chord.

On the left side of the body, the ectoderm is pierced by the exit-tube of the first body-cavity. The centre of the section is filled by the notochord with its large central lumen. Around the cavity is a protoplasmic layer containing nuclei, and the outer portion of the notochord is formed of the clear vacuolated ends of these cells. Below the notochord is the collar-rod, that has been formed, without doubt, by a secretion from the notochordal cells. It is marked by concentric lines of light and dark shading, and these correspond, very probably to alternate periods of rest and activity of the endodermal cells. On each side of the rod, where it slightly bifurcates, lie two spaces nearly filled with large muscle-cells (*b.c.*²). The walls are continuous behind with the walls of the collar body-cavities, and represent forward lateral protrusions from the collar-cavities. The muscles serve probably as the retractors of the proboscis.

Dorsal to the notochord there is in the median line a cavity bounded by mesoderm cells,—the cavity of the proboscis vesicle.

A cross-section of one of the collar-pores is shown in Fig. 78. At this stage, and also in the two preceding stages, the outer wall is turned into the interior of the tube. The cells of this invagination are not so high as are those of the remainder of the wall, and do not seem to be ciliated. Into the hollow of the invagination the wall of the body-cavity is pushed. The rest of the periphery of the organ is also surrounded by a layer of mesoderm. The inturned portion is on the latero-dorsal side of the tube, and the opening or nephrostome is at the inner end of the tube. The groove of invagination is continued backward as far as the external opening of the tube. The structure is wonderfully like a glomerulus.

The nervous system at this stage is larger than in the preceding stages and contains more cells in cross section than in the last case.

The reproductive gonads appeared in earlier stages but the full account was left till the present. *The gonad is formed from the mesoderm.* At the posterior end of the gill-region and closely connected with the ectodermal wall forming the posterior limit of the region the gonads are developed.

An early stage in the development of a gonad is shown in Fig. 79 where the nuclei of the mesodermal wall have begun to enlarge. The preparation is not entirely satisfactory owing to a shrinkage of the mesodermal wall. This is the earliest stage of the gonad that I have found. A later stage is shown in Fig. 80, where the number of nuclei in the gonad is greater and the organ is much larger than before.

In the oldest stage the gonads have considerably enlarged as shown in Fig. 81, drawn to the same scale as the two preceding. A central cavity is forming in the center of the reproductive cells, but as yet the gonad is not connected with the ectoderm.

There can be no doubt that the reproductive cells come from the mesoderm and not from the ectoderm as Bateson thought probable. The gonad is formed as a thickening of the wall of the body-cavity where the somatic and splanchnic layers are continuous with one another. The ectodermal connection of the gonad must come in later.

CONCLUSION.

Growth.

The general phenomena of growth as shown by *Tornaria* present a remarkable series of changes. The larva during the early period of its life is suspended in the water and unusually free from all external agencies that might modify its structure in this way or that. During the earlier stages an immense increase in size takes place. The ectoderm that forms a single layer of cells over the greater portion of the surface has its area many times increased. This is accomplished by the cells increasing in number.

The cavity of the blastocoel space increases greatly. The digestive tract does not increase to nearly the same extent as do the walls of the body, and the result is that the space between

the two walls has a much greater volume. The blastocoel is filled with a gelatinous fluid. This fluid necessarily increases in quantity but does not necessarily retain the same consistency.

I have no observations to show whether the gelatinous fluid becomes diluted as development progresses or not. We might suppose that additional water is taken in so that the ectodermal wall becomes stretched, or we might suppose—in the absence of data—that the amount of the jelly increases. The latter view seems much the more probable, for so far as microscopic examination of dead material goes it shows that the blastocoel fluid is much denser in the older stages. I therefore assume that the fluid is formed by cells of the larvae, but whether these cells are ectoderm, mesenchyme or endoderm I do not know.

The ectodermal ciliated bands increase in size and in the number of their cells and the region of the apical plate is from the start thicker than the general surface ectoderm.

There is a zone of thickened ectoderm found in the earliest stage just in front of the large circular band. This is an important point for, as we have seen, it is in this region and from these cells that the collar region, the gill region and the anterior half of the body proper subsequently develop.

In the very early stages the posterior plate, stretching horizontally across the posterior end of the animal, is a thin membrane. It has thickened when the larva has reached Stage 3 and before the next Stage (4) has become as thick as the ectoderm anterior to the circular band. From this posterior plate is developed the lateral walls of the posterior half of the larva.

We may now pass to the second phase of larval growth during which a continuous *decrease* in size takes place. During this period the proportions of the body change very considerably, but *it is interesting to note that no new organs are formed while the larva is decreasing in size.*

As the size of the larva decreases the ectoderm increases in thickness and although these two changes go on together it must be remembered that the ectoderm continues to thicken after the larva has gone into the sand and when it is again increasing in size. The blastocoel decreases greatly in volume,

and inasmuch as no direct communication with the exterior exists the fluid must either exude mechanically through the walls or else be taken up by the cells and passed out. During this time the fluid becomes of firmer consistency, if one may judge from preserved material. Part of the decrease in size of the blastocoel space may be accounted for by the bulging out of the posterior plate, but only a relatively small amount of the original volume can be accounted for in this way. The body-cavities are enlarging during this period and as the cavities are completely cut off from the cavity of the blastocoel a transudation of fluid must take place into the interior of each body-cavity.

There are many problems of great interest from a mechanical point of view, in connection with the relations existing between the fluids filling a space bounded by cells and the cells themselves. I had my attention called to this problem for the first time when reading Brauer's paper on the development of Hydra. The egg is exceedingly large, but the blastula derived from the egg scarcely larger than the egg itself. All of the protoplasm of blastula is gathered into a narrow peripheral shell, and the center of the sphere is then filled with a large fluid space. The question suggested itself as to whether all of the protoplasm of the egg could be represented in the peripheral shell of protoplasm. It seemed impossible that it could be so represented, and I made an estimate to determine how wide such a shell *of a sphere* would have to be to receive all the protoplasm of the egg—it being understood that a slight increase in size had actually taken place in the embryo. Only approximate measurements could be made in the case cited above, but such as they were they showed that the slight increase in the diameter of the sphere was amply sufficient to accommodate in a peripheral shell all the protoplasm of the egg—a sphere with a slightly smaller diameter. And the increase in diameter calculated as necessary *a priori* corresponded with the actual measurements of the figures themselves. The fluid in the center of the blastula must have passed *through* or *between* the peripheral cells. But although it was possible to account in this way for all of the protoplasm of the egg, it

was still impossible to account for later phenomena. The egg of hydra in the later stages of its development again becomes a solid mass, owing to the filling up of the interior of the blastocoel space by cells from the outer wall. In the case of hydra it is possible, but very improbable, that additional nutriment supplying sufficient material for the new protoplasm might have been absorbed from the walls of the body of the parent hydra. The calculation must be made in other cases where the same process takes place in eggs free from the parent.

In the case of *Tornaria* we cannot make such definite statements as the animal is itself feeding (presumably) during this period, and the larva becomes solid (or nearly so) not by development of any great amount of new protoplasm, but by an actual decrease in the size of the larva as a whole. So great is this decrease that we are obliged to consider that some of the fluid of the blastula space is actually passed out of the animal.

The changes of position that the digestive tract undergoes during this period have been already noticed. The tube itself shortens as its walls thicken, and in order to accommodate itself to the new order of things caused by the bulging out of the posterior plate it is forced, inasmuch as it is attached to the ectoderm at two points, to change its position. It is pulled backwards so that its most anterior point, at first far in front of the mouth, is now carried backwards so that the mouth comes to lie at the most anterior end of the digestive tract.

The general changes in the form of the body have been noticed in the text. The region anterior to the horizontal limb of the longitudinal band becomes the proboscis. The horizontal limb marks the anterior edge of the collar, and the posterior edge of the collar appears a short distance behind in the region lying between the horizontal limb and the large circular band. All of this region is not taken up by the collar, for on the bulging out of the posterior plate the region in front of the circular band has elongated backwards.

The circular band, that in the earlier stage formed a fringe around the posterior end of the larva, comes to lie around the middle of the elongating body of the young worm; it serves as

a landmark by which we are able to determine the subsequent growth of this region.

This brings us to the third period of growth, where the body again begins to enlarge. The enlargement here is brought about in a different way. *The increase in size takes place in all the organs at the same time, and not as in the early stage, at the expense of one set of structures alone.*

The animal *grows* by the increase in size of all the organs of the body. During this period of growth new organs again put in their appearance, noticeably the collar-pores, gill-slits, notochord, and nervous system. There is one fact that has been clearly seen in the later period of growth of the young *Balanoglossus* that I regard as of capital importance: *The increase in length of the young worm is due to a general interstitial growth; the elongation of the posterior metamere of the young worm is not due to apical growth.* We are able to make this statement positively, and I think it must be accepted without question. The position of the circular ciliated band can be followed, as already described, into the later stages, and by this means (taken in connection with the general phenomena of growth) we may make the definite statement given above.

An interesting comparison might be made, I think, as to the method of elongation of the body in such groups as the Vertebrates, Annelids, Nemerteans, and Holothurians. The data pointing to an apical elongation in the Vertebrates and Annelids rests on a sufficiently firm foundation. How much, in addition to this, an interstitial increase helps in the elongation, has not been studied, I think, with sufficient care. In the Nemertians and Holothurians, I do not know of any data sufficient to warrant a definite statement.

In an earlier paper, I have expressed my opinion that the elongation of the body of *Balanoglossus* posteriorly is a secondary growth, and has appeared as a more recent adaptation to a life in the sand; and it is connected, perhaps, with the fact that large quantities of sand are taken into the digestive tube with a relatively small return of digestible material. The immense elongation of the last pair of body-cavities is merely then due to the vegetative elongation of the posterior metamere of the body.

Metamerism of Balanoglossus.

To those who accept the chordate affinities of *Balanoglossus* the question of metamerism of the Enteropneusta is full of interest. But even leaving the phylogenetic question out of account, the problem is of importance in itself. Our ideas as to metamerism have been largely based on the typical metameric structure of Annelids and Vertebrates. And, more or less closely bound up with our conception of metamerism in these groups, the budding theory has played no small part. It is true that other views have been advanced regarding metameric structures as due to the mechanical motions of the body. To state the two opposing theories briefly, if somewhat crudely, the former starts with a short animal and elongates it by serial repetition of itself (budding), while the latter, taking an elongated animal, breaks it up into a series of smaller but similar subdivisions.

For myself, I think a much broader view must be taken of the subject, but the time is not yet ripe for new theories, and what we need most is more facts. It is not my intention, therefore, to go any further into the subject at present, and what I have already said is to prevent misconstruction of what I shall say in regard to *Balanoglossus*.

I regard *Balanoglossus* as a form having three pairs of body-cavities, each pair separated in the dorsal and ventral lines by *longitudinal mesenteries*, supporting (at least for the second and third pairs) the intestine.

The pairs of body-cavities are separated from one another by *transverse septa*, with double walls. This is more apparent for the *transverse septa* between the collar-cavities and the last pair than between the collar-cavities and the first pair.

Now, in both of these respects the structure of *Balanoglossus* agrees with two very important features of Annelids and Vertebrates. Even the external divisions of the body correspond with the internal divisions into metameris, and of course the muscle-system agrees with the same arrangement. Now, I fancy if *Balanoglossus* were shortened, — the proboscis changed into a præ-oral lobe and the posterior

region of the animal made the same length as the collar, — no one would doubt that we were dealing with a metameric animal of three segments. And if we look upon *Balanoglossus* as a form profoundly modified to fit it for life in the sand, if we regard the proboscis as a præ-oral lobe (and head-segment), enlarged as a boring organ, and the posterior metamere of the body immensely prolonged, we cannot escape the conclusion that even in the narrowest definition of an animal with metameric structure we must include *Balanoglossus*.

Collar-pores and Gill-slits.

Several important questions come up in relation to the formation of the collar-pores and the gill-slits. Spengel's statement that the collar-pores form from the first gill-slit cannot be accepted. It would be as easy to maintain the converse, *i.e.*, that the gill-slits come from the collar-pores. We may formulate this statement: *The collar-pores and series of gill-openings arise from a pair of invaginations of the ectoderm.*

This puts the problem in another light, and the following question is suggested: *Is the pair of invaginations to be looked upon as a beginning of an atrium?*

I am not prepared to say that this is true, but it is a view that is suggested by the facts of development, and it seems worth consideration.

From the anterior wall of the invagination a forward extension opens into the collar body-cavity to form the collar-pores; farther back the first gill-pouch of each side opens into the invagination. At a later period the second pouch opens into the invagination behind the first opening, and the succeeding pairs follow the same law. Now, if the backward extension of the ectodermic invagination had pushed back beneath the surface ectoderm, and into this pocket on each side the gill-slits had opened, there would have been little question as to the *resemblance* between such a structure and the formation of the atrium in the *Ascidians*. The possibilities do certainly exist in *Balanoglossus*, but who can say whether they were realized in the higher groups? One observation ought to be noticed here in this connection. The lateral walls of the

invagination can be brought over the external openings of the gill-slits, so that they no longer can be seen in surface views of the young worm. This was seen to happen repeatedly in the living animals.

The serial repetitions of the gill-slits of *Balanoglossus*, *Ascidians* and *Amphioxus* is interesting, although it does not necessarily indicate a phylogenetic connection. Each may have started with a single pair and pursued the same path independently. At present we cannot answer the question positively in either way. On the one hand we find in the most astonishing places external openings between the endoderm and ectoderm. In certain jelly-fish we find such communications at *regular* intervals around the periphery of the bell. In the Mollusca, in Nudibranchs, we have regular openings at the ends of the dorsal papillae. In the Actinians, the Cincelids. Perhaps the anal opening belongs to this category. Similar fusions between the ectoderm and mesoderm are also to be found, as in the serially arranged dorsal-pores of certain Annelids. All this goes to show, I think, the possibility of independent origin of the *series* of gill-slits.

On the other side there stands one fact that seems to me of greater weight than the negative evidence of such general considerations. I refer to the presence and structure of the supporting-bars of the gill-slits. The structure of the supporting-rods of *Balanoglossus* and *Amphioxus* shows an astonishing agreement. In each, chitin-like rods are present in the between-bars (primary bars) and the tongue-bars (secondary bars). In each the rods have a double origin, both for the primary and secondary bars. In each the rods are united along the dorsal edge of the gill-slits, but remain free below. Spaces that seem to be blood-vessels lie at the angles formed by the union of the rods. The body-cavities in both forms extend throughout the length of the bars.

Certain minor points of difference are to be noted. According to the figures given by Lankester the chitin-like rods unite with one another along the length of the bars, the union being closer in the tongue-bars. In the *species* of *Balanoglossus* described here only the rods in the between-bars unite into a

single rod. The second point of difference follows as a corollary to the last statement. In *Amphioxus* the rods unite with one another at the dorsal edge of the gill-slits, forming a *continuous* series, while in *Balanoglossus* on account of the rods in the tongue-bars remaining separate a *broken series* of double inverted U-shaped structures is formed.

A careful examination of the basket of chitin-like rods of *Amphioxus* and *Balanoglossus* will, I think, lead to the conclusion that the structure has developed as a supporting framework for a *series* of gill-openings. If, on the other hand, we supposed a single pair of gill-openings in the ancestral form, and each opening supported by a double U-shaped rod, and, further, that the whole structure repeated itself again and again, still it seems highly improbable that, in the first place, the *double rod* would have appeared in each primary bar, and, in the second place, that the repetition could have taken place in the same identical way both in *Amphioxus* and *Balanoglossus*. It seems to me that we can draw but one conclusion from these data, *viz.*, that both forms have come from a common progenitor with serial gill-slits supported by a chitin-like framework, and that this same structure in a more or less modified form is also inherited by the Ascidians.

If this be granted it follows: That, since it is *the last metamere* of the body of *Balanoglossus* that carries the series of gill-slits and its supporting basket, this *region corresponds to a great number of metameres* of *Amphioxus*. Therefore, either *Balanoglossus* has lost its posterior metameres, for which there is not the least evidence, or else the large posterior metamere of *Balanoglossus* has become split up into several metameres in *Amphioxus*. Not for a moment do I suppose one form to have developed into the other, but that this process took place in some form in the phylum that has led from the common ancestor up to *Amphioxus*. If the validity of this reasoning be granted, we have reached a conclusion of importance for the evolution of the problem of the metamerisation of *Amphioxus*.

Comparisons with Other Forms.

Although short descriptions have been given of several other Tornaria, only in two cases has a sufficiently detailed study been made that is of use to us for our present purpose. Metschnikoff ('69) studied in surface views the transformations of the Tornaria of the Mediterranean, and although his account is accurate as far as it goes, he was limited to a study of the external transformation alone. A brief note by Haldeman, in the circular of the Johns Hopkins University ('86), calls attention to the similarity of the Beaufort Tornaria to the Mediterranean form. Bourne described ('89) the Tornaria found on the English coast, but this seems to be, from the description, identical with the New England Tornaria, described first by Agassiz, and later by myself.

The two forms studied in greater detail are the young of *Balanoglossus Kowalevskii* of the Chesapeake, described by Bateson, and the New England form, studied by Agassiz and myself. During the past summer of 1892 I was fortunate enough to clear up definitely the relationship of the northern and southern *Balanoglossus Kowalevskii* in respect to the development of the young. In both cases a direct development takes place, and the description given by Bateson for the young of *Balanoglossus Kowalevskii* of the Chesapeake will apply equally to the New England form (as found at Wood's Holl and at Newport). The adult of the New England and English Tornaria is unknown, although young larvae have been found in both waters.

It is not my purpose to enter into a long and minute comparison of the form described in the present paper with the two forms mentioned above—one with a direct development, and the other an indirect by means of the Tornaria. Nor would it be profitable at present to push phylogenetic speculations beyond the limits already reached. I wish, therefore, to confine what I have to say under this head to a comparison between the formation of the body-cavities in the three forms.

Bateson described the proboscis body-cavity as arising from the anterior end of the archenteron. It is cut off from the

digestive portion by a constriction of the walls in front of the mouth. The proboscis vesicle is described as formed from mesenchyme cells at the base of the proboscis. The collar-cavities are said to arise by a pair of lateral evaginations from the archenteron. For the present I accept this account of the origin of the second pair only tentatively. The evidence furnished by Bateson does not seem to me conclusive for accepting his statement. I think the phenomena could be explained by a process of delamination or migration, and a subsequent opening (or perhaps the small openings are artefacts). I venture this only as a suggestion, and it is my intention to test Bateson's conclusions by a renewed study of the early stages of development of *Balanoglossus Kowalevskii*. The third pair of body-cavities, according to Bateson, arise as evaginations of the hind portion of the archenteron. To summarize: *All of the five body-cavities of the embryo of B. Kowalevskii arise as enterocoels.*

Agassiz did not study the development of the body-cavities in the New England Tornaria. In my own paper, written in 1891, a great deal of attention was paid to the method of formation of the body-cavities. The origin of the proboscis body-cavity was not seen; and, indeed, no one has as yet clearly made out the origin of this pair of cavities. Götte's account I believe to be due to a misinterpretation, and Bourne's statement that it is formed from scattered mesenchyme cells I cannot accept as proven. On *a priori* grounds, we might accept equally well the evagination of the body-cavity described by Götte, or the mesenchyme origin of Bourne; the evidence for either has not been published. The proboscis vesicle arises, as I believe, from mesenchyme cells, although Spengel has assigned an ectodermal origin to the organ. The collar body-cavities arise by a proliferation of endodermal cells on each side of the stomach. Whether these cells are simply pulled out of the wall, or whether a delamination takes place, I could not fully decide. The last pair of body-cavities arise as solid evaginations of the last division (endodermal) of the digestive tract.

To summarize : *In the New England Tornaria, the origin of the proboscis body-cavity is unknown. The collar-cavities arise each from a single proliferation of endodermal cells, and the third pair of body-cavities arises as solid evaginations from the endoderm.*

In the Bahama form described in the present paper, the origin of the first pair of body-cavities was not found. The proboscis vesicle seems to arise from mesenchyme cells. The collar-cavities differ fundamentally as to their origin from the methods followed in other forms ; they arise at points far distant from the endodermal tube of the digestive tract, and come from cells resembling in every particular the scattered cells of the blastocoel space. Moreover, they do not arise from a single cell or group of cells, but from many such. These subsequently run together into a single body-cavity on each side of the body. Similarly, the third pair of body-cavities has a mesenchymatous origin from many separate cells of the blastocoel.

To summarize : *The origin of the first pair of body-cavities in the Bahama Tornaria is unknown. The collar-cavities and the last pair of cavities have a mesenchymatous origin, and come from many scattered cells united secondarily.*

In drawing our conclusion, we are obliged to leave out of account the proboscis body-cavity, owing to absence of data for comparison. If we are to regard the proboscis vesicle as a body-cavity to be paired with the proboscis-cavity, we may conclude that it has in all cases a mesenchymatous origin (throwing out Spengel's observation).

The conclusion reached in respect to the remaining body-cavities is an interesting one. *They may arise as enteric diverticula, as endodermal proliferations, or even arise from mesenchymatous beginnings.*

The sharp lines drawn by speculative morphologists as to enterocoels, schizocoels, blastocoels are fading out as the evidence comes in. Phylogenetic speculations of far-reaching and of supposed fundamental import, based on the origins of mesoderm in different groups, are losing their hold as facts accumulate.

Nerve Cord.

The gill-slits and the nervous systems of *Balanoglossus* have given the most important data for a direct comparison between the Enteropneusta and Chordata. The evidence furnished by the gill-slits seems to me to be valid, but I think the comparison between the nervous systems of the two groups needs a more careful analysis than has heretofore been given. Spengel in 1884 and Morgan in 1891 pointed out that, in the forms examined by them, the whole length of the nerve cord was formed by a rolling in of ectoderm, while Bateson had described in 1884 the central part of the nerve cord of *B. Kowalevskii* as formed by delamination and only the ends by invaginations. In the Bahama form there can be no question as to the rolling in of the whole length of the cord and the formation of a central canal throughout its entire length.

In 1887 Harmer described the nervous system of *Cephalodiscus* in Vol. XX of the Challenger Reports. "The central nervous system is developed on the dorsal side of the collar as a mass of ganglion cells and nerve fibres lying outside the basement-membrane of the epidermis. It is, however, continuous anteriorly with a similar development of nervous tissue situated on the dorsal aspect of the proboscis, and laterally with a well developed nerve layer on the dorsal side of the lophophoral areas." In the young bud the nervous system is clearly seen to develop from the collar.

The position of the central nervous-system of *Cephalodiscus* might be compared to the position of the cerebral ganglia of Invertebrates in general, and suggests a direct comparison between the two. It must be remembered, however, that this plate of cells remains in the superficial ectoderm in the adult, and no special advantage is gained so far as I can see by such a comparison. It is preferable to look upon it as a specialization of the ectoderm for nervous functions such as is found in Coelenterates and Echinoderms.

I think no one will doubt the relationship between the nervous system of *Cephalodiscus* and *Balanoglossus*, the former representing the more primitive condition from which

the nervous system of Balanoglossus has been derived. In this respect Cephalodiscus is a more primitive form, and I strongly dissent from Lang's view *viz.*, that the apparent primitiveness of Cephalodiscus is due to its sedentary life. That it has been modified to a great degree to fit it for its present surroundings seems to be true, but over and beyond this it seems to have diverged from the main stem of the Enteropneusta a little earlier than Balanoglossus.

In the preceding portion of the paper I have called attention to the method of development of the central nervous system. The mass of cells lying in the median dorsal line of the collar drops out and is covered over by the ectoderm from the sides. *Subsequently this mass of cells is elongated to form the dorsal invaginated nerve cord of the adult.*

Now, if we looked upon the mass of invaginated cells as a cerebral ganglion, and interpreted the subsequent process as an elongation of this cerebral ganglion to form the nerve cord, we would be on the road to a comparison between the dorsal nerve cord of the Amphioxus and the cephalic ganglia of other forms.

Gegenbaur in his text-book advanced such a view without reference, of course, to the forms under consideration, but the view has met with little favor. Morphologists have shown a preference for inverted Annelids or Nemertean with lateral nerve cords.

I have no serious intention of reviving Gegenbaur's speculation, and have referred to the question in order to introduce it, and because at first sight the behavior of the dorsal cord of Balanoglossus seemed to lend itself to such a view. Two very important facts seem to refute such an *interpretation* of the facts in the case we are considering.

I believe it to be a great error to speak of the *invaginated* dorsal nerve cord of Balanoglossus as equivalent to the whole of the dorsal cord of the higher chordata; because:

The invaginated nerve cord of Balanoglossus stretches through only a single metamere of the body.

The invaginated nerve cord does not give off or receive lateral nerve fibres along its length.

My main point of contention is this: the gill-baskets of *Balanoglossus* and *Amphioxus* are so similar that we must believe them to have had a common origin. On this evidence we may safely rest our conclusion of the relationship of the two forms. Admitting this, I have tried to force the issue to its legitimate conclusion, wishing to avoid the introduction of extreme phylogenetic speculation and to stick to observed facts as closely as possible. The gill-basket in *Balanoglossus* is formed on the dorso-lateral walls of a single metamere, but in *Amphioxus* the similar structure extends through a series of metameres. The conclusion seems to follow that the last metamere of *Balanoglossus* must correspond to the series of metameres of *Amphioxus*.

We find in *Balanoglossus* that only a single metamere intervenes between the first true¹ gill-slit and the mouth, that lies between the first and second metameres. Similarly in *Amphioxus* the mouth lies in the young animal approximately between the first and second metameres, and the first pair of gill-slits is found in the region of the second and third metameres.

Further, we see in *Balanoglossus* that the *invaginated* dorsal nerve cord can correspond only to the anterior end of the nerve cord of *Amphioxus*, and that the *superficial* dorsal nerve-path, stretching through the gill region thence to the end of the body, must be the homologue of the remainder of the nerve cord of *Amphioxus*.

¹ I leave out of account the possibility that the collar-pores may be modified gill-slits.

REFERENCE LETTER.

<i>b.c.</i> ¹	Body-cavity, first.	<i>g.</i> ²	Gill-slit, second.
<i>b.c.</i> ²	Body-cavity, second.	<i>n.c.</i>	Notochord.
<i>b.c.</i> ³	Body-cavity, third.	<i>p.v.</i>	Proboscis vesicle.
<i>f.</i>	Funnel or nephrostome of collar-pores.	<i>t.</i>	Tongue-bar of gill-pouch.
<i>g.</i> ¹	Gill-slit, first.	<i>t.b.</i>	Tube of ectoderm leading to mouth.

PLATE I.

All larvae on Pl. I were drawn to the same scale. Camera outlines. Zeiss, eyepiece 2. Objective AA, with lower lens removed. Drawn from living specimen (except 12).

FIG. 1. Youngest Tornaria stage observed. (Stage I.) Length $1\frac{1}{4}$ mm. Side view.

FIG. 2. Older stage than last. (Stage II.) Side view (more of dorsal surface seen).

FIG. 3. Largest stage of free swimming Tornaria. Length $4\frac{1}{2}$ mm. Side view. (Stage III.)

FIG. 4. Larva decreasing in size. Side view. (Stage IV.)

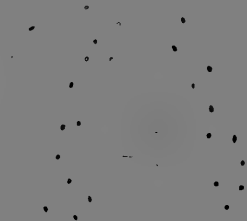
FIG. 5. Transition stage between Tornaria and young worm. Side view. (Stage V.)

FIG. 6. Smallest size reached by older larva. Still free swimming. Dorsal view. (Stage VI.)

FIG. 7. Young worm with two gill-slits. Taken from sand. Dorsal view. (Stage VII.)

FIG. 12. Bimini Tornaria. Side view.

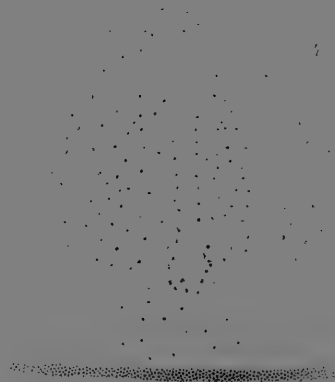
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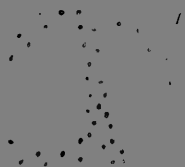
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7



1



12



5



6



PLATE II.

Drawn to same scale as figures of Pl. I (except Fig. 11, which is not so much magnified).

FIG. 8. Young worm with three pairs of gill-slits. Dorsal view. (Stage VIII.)

FIG. 9. Young worm with (probably) four pairs of gill-slits. Only three were seen in the living larva. Dorsal view. (Stage IX.)

FIG. 10. Young worm having probably six pairs of gill-slits. Only three pairs were seen in the living larva. The true number determined by surface views of preserved specimens, and from sections. Dorsal view. (Stage X.)

FIG. 11. Oldest worm obtained from *Tornaria*. Length 26 mm. Side view. (Drawn by Dr. E. A. Andrews.) (Stage XI.)

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PLATE III.

Fig. 1-5: Stage I. Fig. 6-15: Stage II. Fig. 16-22: Stage III.

- FIG. 1. Section of tentacle. *Zeiss*, 2 D.
 " 2. Section of circum-oral region. *Zeiss*, 4 D.
 " 3. Surface view, stomach and first body-cavity, taken from cross-sections.
 " 4. Cross-section of anterior wall of stomach.
 " 5. Cross-section of oesophagus.
 " 6. (Stage II.) Longit. section of lateral body-wall.
 " 7. Surface view of left side of apical plate.
 " 8. Cross-section of stomach and ectodermal tube.
 " 9. Cross-section of stomach and opening ectodermal tube.
 " 10. Cross-section of body; youngest stage proboscis vesicle.
 " 11. Cross-section of body; older stage proboscis vesicle.
 " 12. Cross-section of body; next section to last.
 " 13. Cross-section of body; through third body-cavity and circular band.
 " 14 *a-b*. Cross-section of body; to show origin of third body-cavity.
 Left side.
 FIG. 15 *a-h*. Cross-section of body; to show origin of third body-cavity.
 Right side.
 FIG. 16. (Stage III.) Surface view of extra-oral area. *Zeiss*, 2 D.
 " 17. Surface view of circum-oral area. *Zeiss*, 2 D.
 " 18. Cross-section of tentacle.
 " 19. Surface view of apical plate.
 " 20. Surface view of eye. *Zeiss*, 2 F.
 " 21. Optical section of eye. *Zeiss*, 2 F.
 " 22. Actual section of eye. *Zeiss*, 2 F.

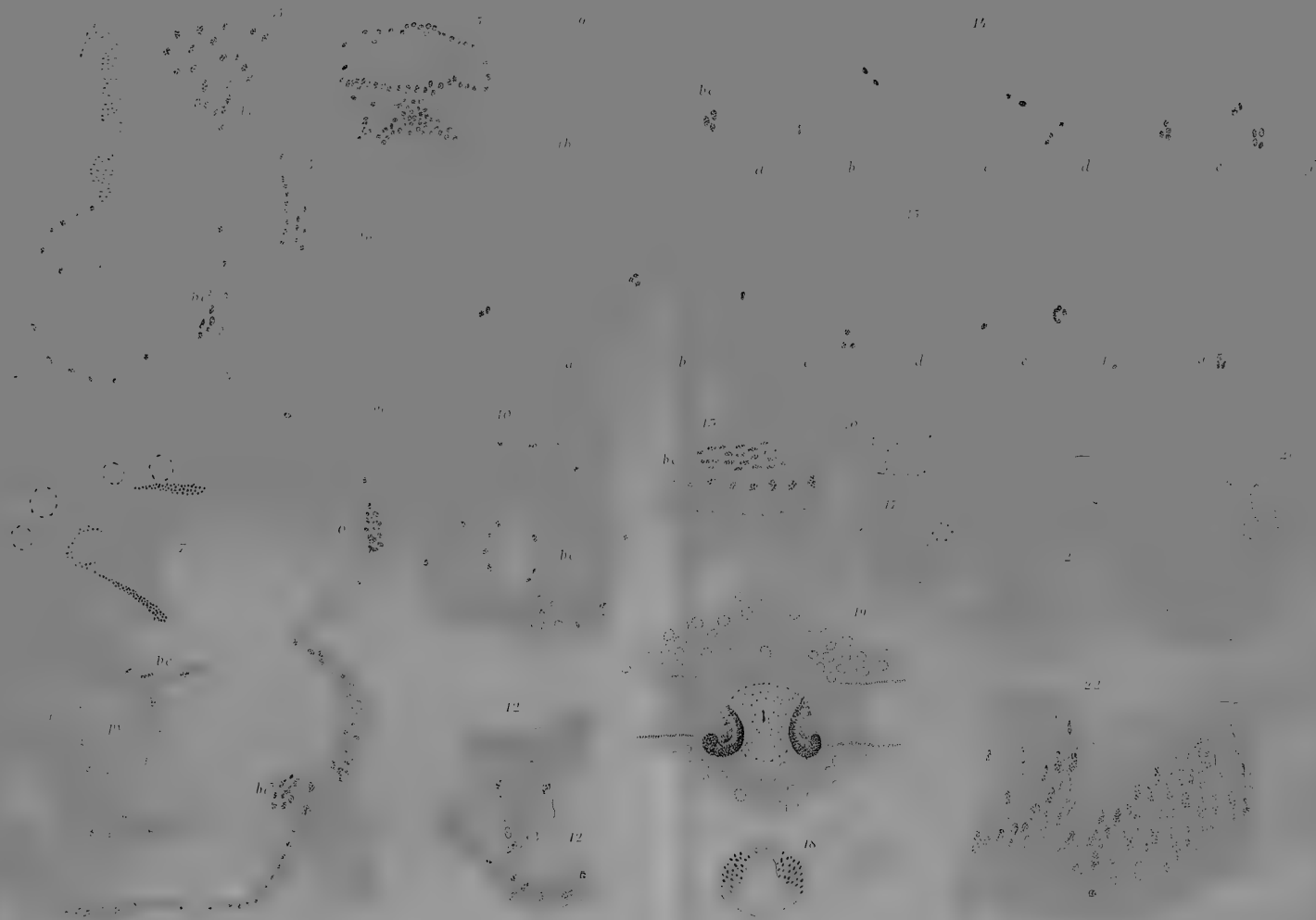


PLATE IV.

Fig. 23-34: Stage III. Fig. 35-40: Stage IV.

- FIG. 23. (Stage III.) Surface view of posterior plate.
- " 24. Surface view of small circular band.
- " 25. Surface view of larger circular band.
- " 26. Cross-section of middle of body.
- " 27. Cross-section of ectodermal tube leading to mouth.
- " 28 *a* and *b*. Cross-section of oesophagus.
- " 29. Cross-section of lateral wall of body.
- " 30. Cross-section of third body-cavity.
- " 31. Cross-section of second body-cavity.
- " 32. Surface view from side of first body-cavity and proboscis vesicle.
- " 32 *A*. Section through last. See vertical line in 32.
- " 33 *A-F*. Section through Fig. 32. See horizontal lines in 32.
- " 34. Surface view (from section) of wall of proboscis vesicle.
- " 35. (Stage IV.) Cross-section of lateral wall of body.
- " 36. Cross-section of tentacle.
- " 37. Longit. section of apical plate.
- " 38. Cross-section of anterior wall of stomach.
- " 39. Surface view of wall of third body-cavity.
- " 40. Cross-section of proboscis wall and first body-cavity.
- " 41. (Between Stage V and Stage VI.) Longit. section of Tornaria.
- " 42. Earliest stage of collar-pores. Longit. section.
- " 43. Two mesenchyme cells of blastocoel.
- " 44 *a* and *b*. Surface view and optical section of eye.
- " 45. Actual section of eyes and apical plate.
- " 46. (Older) surface view of apical plate.
- " 47. Longit. section of wall of proboscis.
- " 48. Cross-section of body proper, (older).
- " 49. Beginning of dorsal nervous system.

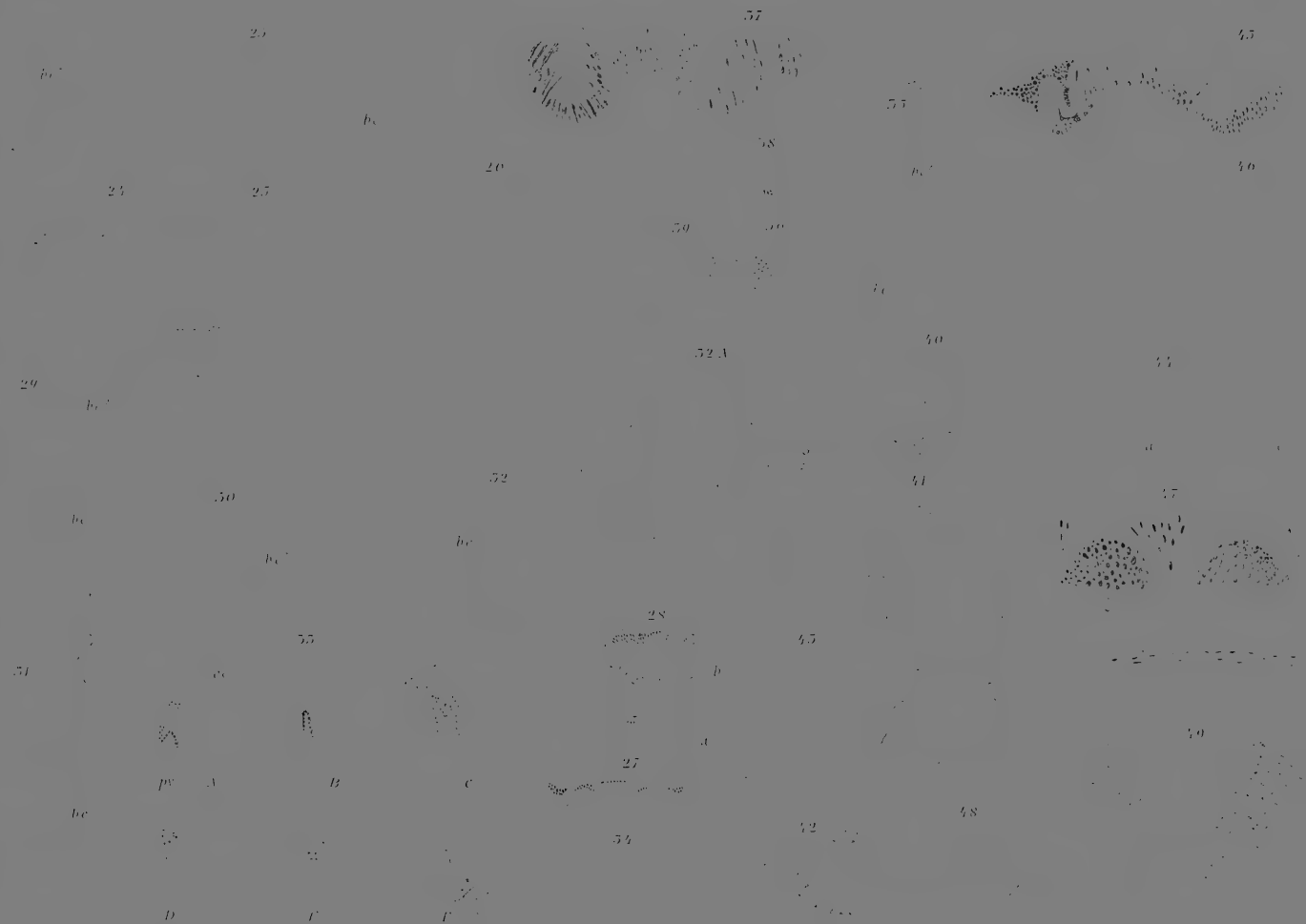
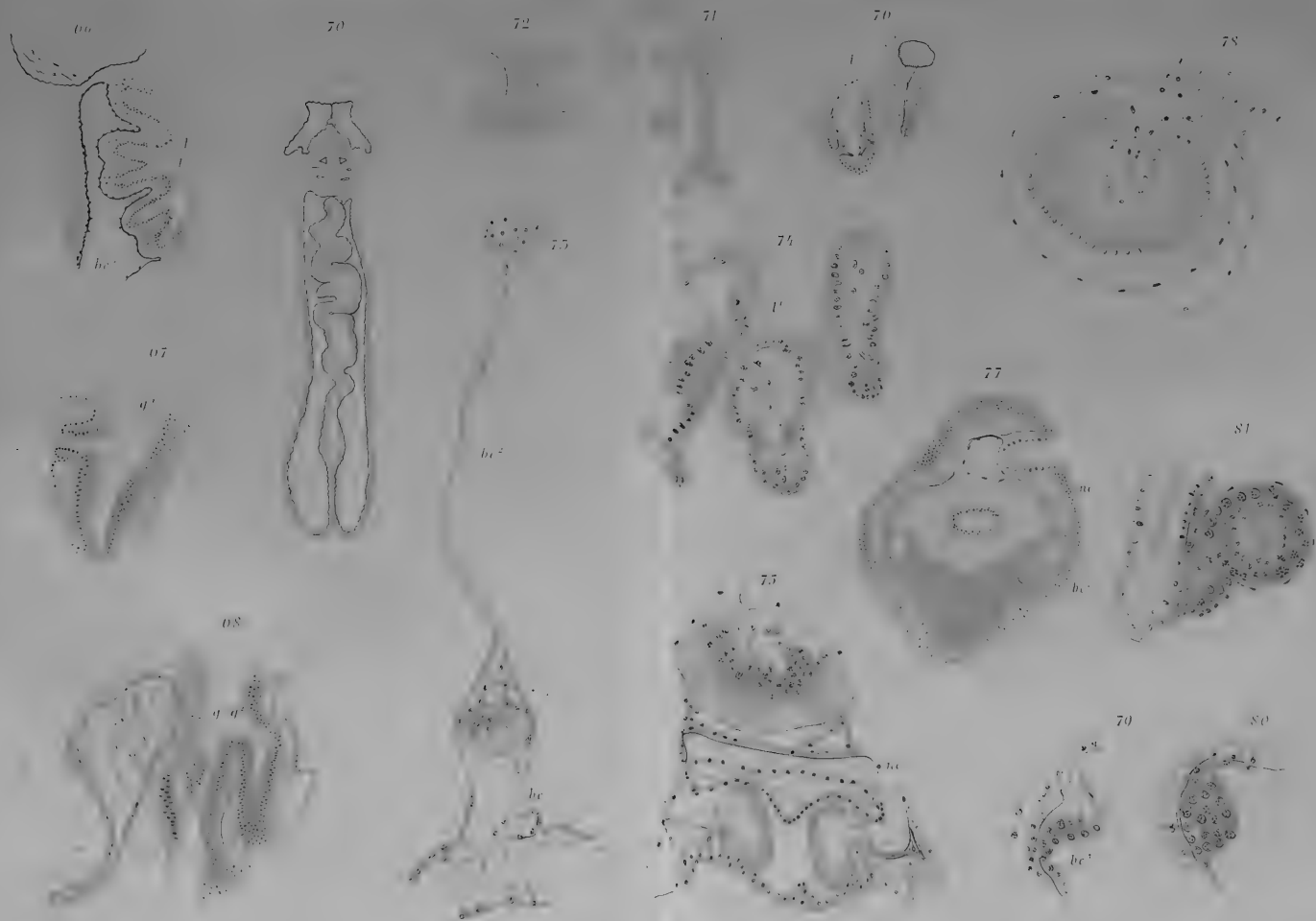


PLATE V.

- FIG. 50. Cross-section of nervous system. Same series with last.
" 51. Surface view of small circular band.
" 52. Second "stage" in development of collar-pores.
" 53. Cross-section of collar.
" 54. Cross-section of proboscis wall and body-cavity.
" 55. Surface view of outer wall of first body-cavity.
" 56. Cross-section of proboscis vesicle, *etc.*
" 57. Surface view of oesophagus with gill-pouches. Lower part figure is anterior.
- FIG. 58. Longit. section of proboscis vesicle, *etc.*
" 59. Longit. section of body. Invagination of ectoderm for gill-slit and collar-pore.
- FIG. 60. Longit. section of body. Opening of collar-pore into second body-cavity.
- FIG. 61. Longit. section of body. Same series as last.
" 62. Longit. section of body. Same series as last.
" 63. Cross-section of nervous system.
" 64. (Stage VII.) Longit. dorso-ventral section.
" 65. Cross-section of nervous system. Older than Fig. 63.
" 66. Cross-section of nervous system. Older than Fig. 65.

PLATE VI.

- FIG. 67. Longit. section, vertical. Opening of first gill-slit, *etc.*
" 68. Longit. section, vertical. Opening of second gill-slit. Same series.
" 69. Longit. section, vertical. Same series, nearer middle line.
" 70. Longit. section, horizontal, of Stage VIII.
" 71. Surface view from within of nerve-cord.
" 72. Surface view from within of nerve-cord. Younger.
" 73. Cross-section of collar to show nervous system, *etc.*
" 74. Cross-section of first gill-slit. (Horizontal series.)
" 75. Cross-section of nerve-cord, notochord, *etc.*
" 76. Cross-section of gill-region. (Horizontal series.)
" 77. Cross-section of neck.
" 78. Cross-section of collar-pore.
" 79. Youngest stage of development of gonad from body-wall.
" 80. Older stage of development of gonad from body-wall.
" 81. Oldest stage observed of development of gonad.



CONTRIBUTIONS TO THE MORPHOLOGY OF CLADOSELACHE (*Cladodus*).

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UNTIL very recent times the Morphology of Elasmobranchs has received few contributions from the study of the earlier fossil forms. It was, in fact, hardly to be expected that uncalcified shark structures should have preserved with any completeness the record of their ancient characters. Fragmentary remains on the other hand have, in many cases, led to confused and contradictory results. It was not until 1888¹ that accounts, in any way satisfactory, were given of the chief features of palaeozoic sharks. In this year there appeared almost simultaneously, a description of the Carboniferous *Pleuracanthus* by Brongniart,¹ of the Lower Carboniferous *Chondrenchelys* and *Cladodus* (pectoral fin) by Traquair,^{2, 14} of a *Cladodont* shark from the Ohio Waverly by Newberry,³ and of the pectoral fin of *Xenacanthus* by Fritsch.⁴ Subsequent papers of especial importance morphologically were contributed by Fritsch,^{5, 6} Döderlein,⁷ Smith Woodward,^{8, 9} Newberry,¹⁰ Wiedersheim¹¹ and Jaekel.^{12, 13}

The sharks discussed at that time by Newberry were received from Rev. Dr. William Kepler of New London, Ohio. They had been collected at Linton, and represented the remains of about six individuals. The remarkable characters they exhibited proved in no small degree puzzling to their describer. The dentition was undoubtedly *Cladodont* and the name *Cladodus* was retained, although it was clearly recognized that a number of genera and even families might be represented by this generalized type of dentition, and that a new genus might be assigned when more material of the type (*Cladodus mirabilis*) should be found. Whether the partial description

¹ The writer must here except the memoir of Cope¹⁵ on *Didymodus* and the earlier studies of Acanthodians.

given by Traquair¹⁴ pertained to *Cladodus*, Newberry was somewhat in doubt. Traquair had ascribed to a shark whose teeth were of the *mirabilis* type, a pectoral fin whose form was a "monoserial archipterygium intermediate between the truly biserial one of *Pleuracanthus* and that of the modern shark."

The writer, basing his observations upon recently discovered material, must, however, follow a suggestion of Smith Woodward as to important differences in fin structure, and regard the shark of the Waverly as entirely distinct from the *Cladodus* of Traquair. Accordingly he regards it necessary to distinguish the American form, and would suggest a new genus, *Cladoselache*.

The material from which this shark type has been studied is in the possession of the museum of Columbia College. It includes the types of Newberry, a number of specimens hitherto undescribed, together with a most interesting and well-preserved example of *C. fylleri*, recently acquired. This specimen is one of an admirable series that Dr. Kepler has succeeded in discovering at Linton, and is the first which has been known to exhibit the tail structure.

At the present time it is possible to consider more exactly the structural characters of this generalized shark type, and they prove of no little interest from a morphological standpoint. The evidence they present as to the origin of paired limbs Smith Woodward⁹ has lately commented upon, regarding their fin structure as the least modified of known forms in which the lateral fold has become divided into its two elements. On the other hand there may be considered in some detail the objections recently urged by Jaekel¹² as to primitive characters. This author, for example, would consider the fin structure as of essentially a modern type, ray-like in its specialization to bottom living, in no way, therefore, strengthening the lateral fold doctrine. He assumes, moreover, that the entire posterior portion of the body in the type specimens, which he as well as Smith Woodward had examined in the museum of Columbia College, had been falsely added; although he admits that the presence of circumorbital derm plates is of especial interest phylogenetically (*Acanthodian*).

In the arrangement of his paper, the writer has for convenience, considered questions of relationship under structural headings.

THE AXIAL SKELETON.

The notochord was probably persistent and its surrounding tissue but little calcified. In the trunk no traces of its structures have been found. Its caudal termination is, however, well shown; in this region its substance is compact, finely granular (Fig. 3), lacking the sheath constrictions of *Pleuracanthus* and *Chondrenchelys*.² In the upper lobe of the tail the chorda narrowly tapers, and extends to the extreme tip. As will be later noted, the hypural rod-like supports are here lacking, replaced by an unjointed sub-notochordal rod of semi-calcified cartilage. Of the neural canal in this region little can be learned; its size must have been exceedingly minute, judging from the close origin of the epural supports to the notochord. From the type of chord thus suggested it is not unreasonable to infer that the vertebral arches, of which no traces have been found, must have been of the simplest type, certainly as primitive as those known to occur in *Hybodus*. In no part of the axial skeleton has the writer found the coarse beaded calcifications typical of the 'Ichthyotomi.' In general calcification appears centripetal, is marked in basal fin rays, jaws, skull and shoulder girdle. The head parts appear generally similar in character to those of *Chlamydoselache*. There is no evidence of what were described by Cope as cranial elements in the Permian *Didymodus*,¹⁵ nor of the "parasphenoid" of *Chondrenchelys*. The mouth was terminal, not as large proportionally as that of *Chlamydoselache*. The shape and disposition of the mandibular rami indicate a narrow and pointed snout; at the symphysis, however, their proportions appear to have been heavier than of *Chlamydoselache*. Relatively the nares and eye capsules were slightly further forward. The orbit could have been little anterior to mandibular articulation—and suggests, therefore, a short down-turned suspensorium. From present material there is no evidence of a post-orbital facet for

the first arch. The number of the branchial arches cannot be definitely stated. In one example five gill-slits are to be determined, and a sixth and seventh are certainly suggested. The arches are directed sharply caudad and their distal (ventral) ends could not have been widely separated. The anterior arches were the stoutest and largest; the foremost pair of slits appears to have been connected ventrally by a loose isthmus-flap as in *Chlamydoselache*. In a number of specimens the branchial region is marked by a series of parallel lines, usually transverse, suggestive of fibrous filament bearers, or more probably of supports for loose branchial-flaps. This peculiar character of striation is certainly found in no other portion of the fossils than in the branchial region, *i.e.*, anterior to pectorals and posterior to the region of mandibular articulation. The laminae appear to have been long, and possibly favor the deduction of Garman¹⁷ as to the protrusion of gills in the ancient Cladodont.

THE PAIRED FINS, AND THEIR SIGNIFICANCE.

The mode of origin of the paired fins, as most recently stated by Wiedersheim,¹⁰ is in no little way explained by the fin-type of the Carboniferous and Permian genera, *Xenacanthus* and *Pleuracanthus*. In view of the studies upon these forms, the fin-structure of *Cladoseleche* becomes of especial interest as clearly a more primitive form. Wiedersheim, commenting on the studies of Döderlein and Fritsch on *Pleuracanthus* and *Xenacanthus*, notes in summary that the structures of the pectoral fin of these forms are equivalent to those of the ventral of the most primitive of recent selachians (the structure of ventral being of course more primitive than that of the corresponding pectoral), and shows that the ventral in these fossil genera illustrates the simplest of known conditions in a paired fin. In *Cladoseleche* present material shows that the pectoral indicates a more primitive condition than even the ventral of *Xenacanthus*, and offers in a most remarkable way actual proof of the proposition that the ventral in *Xenacanthid*, arranged after the uniserial type, demonstrates that the pectoral too must originally have been of this form.

Smith Woodward⁹ has reviewed the origin of the paired fins in the light especially of the more recent work in palaeontology. He derives them from the crowding of the fin-supports at two definite points, with a tendency towards the contraction of the base of the fin to its narrowest limits, with a subsequent broadening out of the basal portion to become in the pectoral tribasal or polybasal. It is interesting that he has taken the fin of *Cladoseleache* into especial account, regarding it as exhibiting one of the least modified conditions of the exoskeleton of the lateral fin-fold that can be expected in any fish in which this fold is already subdivided into its ordinary two remnants. In the pectoral "no fin-basals can be detected with certainty in any of the specimens the writer has examined; and none of the cartilaginous rods that support the fin-membrane are transversely jointed. The most singular feature of the fin consists in the evidence it affords of that crowding and concentration we have already observed in the differentiated median fins of the earlier fishes. Between the extremities of the unaltered parallel bars there are the remnants of similar bars that have evidently been reduced and displaced by growth pressure. Most of the cartilages bifurcate a little distally, but that is a minor matter. The segmentation of the rays, the persistence of one of the middle rays, with the concomitant partial fusion of the still further crowded and reduced bordering rays, would soon, in the writer's opinion, result in the 'archipterygium' of Gegenbaur. It is, moreover, significant that the anterior (preaxial) rays are much more robust than the posterior (postaxial) rays, exactly as in all known examples of 'archipterygium.'"

In this interpretation, however, Smith Woodward is opposed by Jaekel, who had also examined the specimens in the museum of Columbia College. "There is no doubt that the outer clearly-marked fin rays take their origin and diverge from an inner basipterygoid." These rays, he continues, have been mistaken for the supports of an older continuous fin fold. The pectoral fin characters are rather to be reduced to the type of *Xenacanthus*; it is but a *Convergenzerscheinung* of a bottom-living Selachian. The fins, like those of *Xenacanthus*,

are resolvable into a double row of cartilage rods; their structure is the outcome of their living conditions. He thus concludes that *Cladoselache*, if not falsely restored, would be a typical selachian, possessed of all the essential peculiarities of its later kindred.

Before these contradictory views may be considered, the structure of the paired fins should be re-examined in the light of the newly-discovered material.

The pectoral fin (Fig. 1) is remarkable in that its broad base is continuously attached to the body, and that its plane is in the moving direction of the fish. It is a flat triangular plate, directed outward and slightly downward. There can be no doubt that the web-like posterior fin margin was continuous with the trunk integument, and that there was no projecting tip of a lateral Stammstrahl such as Fritsch has figured in the ventral of *Pleuracanthus*, or such as Traquair¹⁴ has described in the pectoral of *Cladodus*. The figure shows clearly the row of parallel cartilaginous rods remarked by Woodward, that appear to take their origin in the outer body wall and proceed directly to the outer fin margin, unjointed. The rays may, for convenience, be divided into three groups of about ten each,—fore, middle, and aft,—which in structure grade imperceptibly one into the other. The first group is of stout bars of cartilage, and includes about half of the fin; its foremost ray is the stoutest, shortest, is directed forward; and the following rays, increasing in size and compacting together, form the stout anterior margin of the fin. The middle rays are the longest, tapering and often forking at the tips. The rays of the final group are narrower, slenderer, and more forked; they decrease in size, becoming more and more posteriorly directed. Noteworthy is the clustering process which these rays appear to be undergoing, as pointed out by Smith Woodward; their bases are so tightly crowded together that the rays have been obliged to form a dorsal and a ventral set, as may be clearly seen at the distal end of the fin where the flattening has given room for the tips of the alternate rays to assume their position in a single plane. The strength of this compact structure is noteworthy; clustered

rays, stout at the base, whose free tips intercalate to weave a stout fin margin. In the posterior fin margin bifurcated tips, as we have seen, often interwedge. Compactness of supporting elements has the effect of making the fin appear thick and immovable, and the inclination of the compressed foremost rays tends to give the entire fin the appearance of being directed forward.

As to the attachment of the fins to the body,—basalia and shoulder girdle: from the specimens earlier discovered but little was to be learned. Newberry asserted the presence of basal plates; but of this interpretation of the fossil Smith Woodward, as noted above, was skeptical. It was clear that the origin of the laterally-placed fin was stout and muscular, giving the latter authority, no doubt, an additional reason for his conclusions as to the lateral-fold character of the fin. Newly obtained specimens, however, showed the present writer that basal plates are unquestionably present, and by careful comparison of his material he was enabled to prepare the accompanying figure (Fig. 1) of the shoulder girdle with its basalia, which he later found, from Fritsch's drawings, readily comparable with the pelvic structures of *Xenacanthus* (female)

There is certainly a series of cartilage plates (basalia) from which the rays take their origin. These, however, are broader and more of a size than those of *Xenacanthus*. They lie within the body wall, and the distal element does not protrude. What in the diagram is termed basal (*Bas*¹) may not improbably correspond with the element proximal to that lettered *Bas*¹ in Wiedersheim's text figure 5a; Basalia one and two are certainly of great interest as possessing coalesced and disappearing fin rays; their arrangement is certainly such as might be expected on the principle of fusion of proximal elements, and clearly suggests the gradual inturning of the anterior end of the line of the basalia. It is further instructive in the general principle of fusion that the proximal basal elements are smaller and decrease in size as they come to be turned towards the median line. Although the present material allows a fairly complete idea of the area and position of the distal basalia, the separation of the caudad elements

must be regarded as doubtful. They are, however, suggested as the writer has indicated in the diagram with dotted lines. Their fusion into a single plate would not prevent them subsequently from becoming conveniently separated or receiving distal increments where their axis comes to protrude from the body wall.

The basal joints of the foremost fin rays may be significant as the first appearance of jointed cartilage rays in a vertebrate extremity—their positions, flanked by an unshifting wedge of cartilage, might seem to suggest a reason for their flexibility and therefore for the origin of the joint. May not the condition here in its beginnings present the advantages of capacity for fusion and for flexibility, which will later be transmitted to the remainder of the fin, and give rise to the stage of its evolution figured by Wiedersheim? It would further appear that the inordinate forking, crowding and coalescence of rays in distal elements would naturally be most marked in the median and hinder portion of the fin when the distal end of the axis (basalia) has come to be external.

It is certain that in *Cladoselache* the fin rays (radialia) in their (unjointed) primitive character proceed directly from body wall to fin tip,—while in *Xenacanthus* and *Pleuracanthus* these have become jointed, often fused by lateral pressure, and reduced proximally to such a degree that more than half of the fin is dermal.¹ Derm rays have certainly, even in these early forms taken the duty of the marginal cartilage rays. It is significant that the cartilage rays in the posterior portion of the fin of *Cladoselache* become delicate and fork in their efforts to combine flexibility and lightness with strength.

It is to be noted that this region when the fin margin becomes membranous delicate striation may be seen,—these lines are parallel to the direction of the fin rays, pass between them, and represent perhaps, the beginnings of dermal rays which margin the fin in *Pleuracanthus*.

The characters of the pectoral of *Cladoselache*, thus described, would accordingly represent a more primitive condition than

¹ 'Dermal' is here used mainly for convenience, for in ontogeny these fin rays have been shown by Ryder to be mesodermic.

that even of the ventral fin of *Xenacanthus* (female). The axis of the basalia in the latter has already emerged from the body wall, has become of the archipterygial type, clustering its radials distally in what Fritsch and Wiedersheim call the *post-axial* Strahlenreihe. This fin type will shortly (as in the pectoral of the same form) proceed to form the biserial archipterygium by the process of ray splitting being continued from the tip to the opposite side (pre-axial of Fritsch) of the fin axis.

One would now naturally look to the simpler structure of the ventrals of *Cladoselache* for further light on the primitive vertebrate extremity. One of the best specimens of these has already been figured by Newberry,¹ but may here (Fig. 2), with slight modifications, again be figured.¹

The ventral is first to be noted as a longitudinal fin nearly three times as long as wide, its length alone suggestive of its archaic fin-fold character. Its plane is that of the pectoral; it is more delicate, and smaller, less by two-thirds in linear measurements; its position is midway between pectoral and caudal. Like those of pectoral its rays are unjointed, proceed from body-wall to fin-tip; the foremost are the smallest, stoutest and most clustered. Unlike the rays of the pectoral they never branch, are distinctly and sharply tapering, show no specialized shapes, are directed somewhat backward, and vary but little in their angle of inclination, save that the posterior rays are directed slightly further backward. The ray concentration at the anterior margin is regularly accomplished. In the median portion of the fin nearly the entire length of the alternating rays, *i.e.*, those whose bases have been compressed into the ventral fin-surface, is to be seen. The sloping, anterior fin-margin is exactly what might be expected if the intervening fin-fold be imagined to have slowly disappeared; its slow disappearance might also account for the concentration of the rays in the anterior margin, whose function now must include that of cut-water. The hind portion of the fin, naturally least modified, seems little more than the actual remnant of

¹ In no specimen are claspers present; sex apparently is not to be distinguished by the shape or character of ventrals.

the fin-fold of the ancestral vertebrate. The anterior fin-margin, like that of the pectoral, is encrusted with denticles of shagreen.

The supports of the ventrals are entirely in accord with the primitive disposition of the fin-rays. The basalia, which in *Xenacanthus* and *Pleuracanthus* have coalesced, are here in a condition of concrescence in the anterior root of the fin, *but are still separate, one from the other, and are still rod-like, homologous in every way with the baseoste of an unpaired fin.* A still more proximal element has not been determined, but it may not unnaturally be inferred, from the very convergent nature of the rod-like basalia, that a pelvic cartilage, — if one existed, — must have been exceedingly small, representing the coalesced axonosts of a paired fin of so azygous a type. It is with some doubt that dermal striation is to be observed in the ventrals.

Returning to the view of Smith Woodward as to the significance of the fins of *Cladoseleache*, it will at once be seen that the discovery of basalia must of necessity modify his general conclusions. The concentration of the rays in the anterior and median portion of the fin might not imply that the process of joint-forming, and of the out-sprouting of an "archipterygium," would here take place; it would seem that these conditions would rather arise in the regular train of evolution exemplified in *Xenacanthus* and *Pleuracanthus*. Concentration of rays would appear a modification of the anterior rim of this primitive fin as a support or cut-water. The compressed nature of the fin-rays (*i.e.*, there appearing to be two layers, the tips of the under layer being only seen) might, moreover, be regarded as a specialized device for strengthening the fin-plate, unless one were to devise an unnecessary theory as to the original derivation of paired fins from double lateral folds. The primitive character of the paired fin, the significance of the radial cartilages, and the manifest homology of paired to median fins, the recent material shows that Smith Woodward has very precisely indicated.

Jaekel, on the other hand, has taken a most conservative view as to the morphological value of the fins of '*Cladodus*.'

He finds in them, if anything, an argument against the lateral fold doctrine. He regards *Cladoselache* as "a typical selachian possessing all the essential peculiarities of its later relations." That the fins were not, according to his interpretation, of dermal fold character is fatal evidence against the atavistic value of the early lateral folds of *Torpedo*.¹⁸ "From the structure of its (*Cladoselache*) pectoral we shall find no possible ground for deriving the paired limbs from lateral folds," but at the same time he admits that there is no ground for asserting the presence of the "archipterygium" of Gegenbaur, and notes that it is of the plan of structure which Fritsch has shown as the stem-form for the paired fins of Xenacanthids, a group whose specialized archipterygium is evolved from its conditions of living. *Cladoselache*, he concludes, shows in the structure of pectoral nothing more than would be seen in an immature fin condition of modern sharks. The rays of the ventral joined to basal cartilages are adduced as an additional ground for the ungeneralized nature of the paired fins.

At the present time the writer would regard the results of Jaekel as hardly to be warranted. Aside from the general primitive characters of the *radials*, as pointed out by Smith Woodward, we now are able to determine from the basalia of the pectoral direct homologies with those of the ventral in the Permian forms,—we find indicated in the ventrals not the "Flossenstrahlen" and "Knorpelspange" that Jaekel uses as an argument for its modern type of structure, but *primitive, unjointed radialis* and *basalia that are as yet altogether unfused*. In this remarkable basal condition the fin would at once seem more primitive than that of the cartilaginous ganoids,—which Wiedersheim states is below that of selachians, excluding Xenacanthids: the latter, he states, present the most generalized characters because they were lacking in hip girdle, show the fused basalia, and present as many as twenty radials.

Cladoselache now becomes of interest, appearing to foreshadow even these primitive characters. In its ventral it possesses 22–23 radials, as many as nine unfused basals, arranged in a way suggestive of lateral fold, with no axis

protruding from the body wall ; the arrangement of the basalia, moreover, gives ample ground for regarding a girdle as lacking.

That *Cladoselache* is by no means of a modern order of shark Jaekel, himself, has already given one and a very convincing proof, in describing the circum-orbital ring of derm plates, whose acanthodian value might at once have led to a closer scrutiny of fin characters. A final and positive proof as to the claims of this fish to archaic characters is now at hand in the unique structure of the caudal. Jaekel has several times referred to the "painted tails" of the older specimens, figured by Newberry ; these now prove, from an examination of a number of examples, to have been in no way falsely restored—if restored they had ever been—by their collector. The spade-like body terminal, as it was originally figured, appears now to have represented a vertical projection, whose tapering apex represented a portion of the margin of the tail. The length of the specimens (which Jaekel states should be considerably greater) proves to be exactly as Newberry described it.

THE CAUDAL OF CLADOSELACHE.

The structure of the caudal was a remarkable one,—its characters strongly suggestive of *Acanthodes*. The notochord extends to the tip of the upper lobe in the usual elasmobranch manner, but is so sharply upturned that the tail has become widely vertical (Fig. 3). So broadly have the lobes forked that the tail outline has become that of *Xiphias* ; its total breadth equals the measurement from tip to tip of the pectorals. Its posterior margin is not, however, an indented one ; it forms a straight line at right angles with the axis of the fish.

Structurally, the remarkable character of the fin is that the upper lobe is strengthened *only on the neural side* ; it is wanting in hypural rays, and is in this region web-like. Epurally it is supported by a prominent cut-water of well-defined cartilages.

In hypural characters the caudal structures may well be compared with those of *Acanthodes* ; there is a series of

stout parallel rays of cartilage which form the inferior lobe of the tail, but disappear in the hinder web midway from tip to tip. In *Cladoselache* these are about twelve in number, the middle pair being the longest, the remainder extending in graded sizes to and from the lobe tip. They are unjointed; of their connection with the haemal arches no satisfactory determination may be made; it appears, however, that they were attached at no great distance from the notochordal sheath. Rudimentary structures pass caudad; four are seen to be separable as basal supports; a terminal cartilage bar is closely apposed to the chord.

Unfortunately the upper lobe of the tail cannot be compared with that of *Acanthodes*, since the epural structure in the latter is obscured by the crusting remains of shagreen. In the present case nearly two-thirds of the lobe breadth is formed of a compact row of epural cartilages. These supports are readily to be reduced to proximal and distal elements, and indicate as well traces of a second proximal division, making the entire epural structure comparable in its elements to radalia, basalia, with perhaps included axonosts. Of the distal elements about fourteen may be defined, and appear to be curiously homodynamous with foremost radials of the pectoral; thus the foremost are the shortest and stoutest and are directed forward, blunt ended, while those succeeding come to be gradually elongated and directed more and more caudad. The row of basal elements is less readily separable, seven plates perhaps may here be included, although doubtfully; and of the proximal row nothing is positively definable.

The membrane-like posterior margin of the caudal extends between the tail lobes in a straight line from tip to tip; in the upper lobe it reaches proximad to the sub-notochordal cartilage; in the lower it gives the rays a distal derm margin of about $\frac{1}{4}$ inch. It is particularly interesting to note that here again appear the beginnings of dermal rays (trichinosts) extending in the same direction as indicated in recent forms. They are so fine in character that they are scarcely to be seen by the unaided eye (240 to an inch). They exhibit no branching or jointed structure. The actual

margin was slightly crenulate, and bears a coarser type of granular shagreen than that which covered the entire tail. The tail's anterior margin, like that of the other fins, was coarsely shagreened.

There can be little doubt that on either side of the base of the tail there was present a longitudinal derm fold or keel, not unlike that developed in a number of recent fish forms whose caudal outline is similar to that of *Cladoselache*. In specimens whose ventral aspect is preserved (and these are in the majority of instances) the flattened body terminal, exaggerated doubtless by mechanical causes, spreads out like the fluke of a sirenian, the tail itself to be seen in vertical projection as an acutely produced apex; the outline has in these instances been formed by the lateral keels. That this keel was a stout one appears probable, as traces of it are to be found in examples which have preserved the lateral aspect of the tail.

The significance of the caudal of *Cladoselache* is not readily to be determined. At first sight its apparent specialization is not at all in keeping with the peculiarly archaic and generalized character of the paired fins. Its broadly heterocercal tail has appeared to have reached almost the limits of homocercy, — it certainly *seems* more specialized than the caudal of Xenacanthids. The peculiar nature, on the other hand, of the epural supporting plate, and of the dermal margin of the upper lobe leads one naturally to the closest examination of suggested relationships, homodynamous, to paired fins.

The type in this early fin is certainly a pure heterocercal one, in no way approaching the archaic diphyrcy which one might naturally expect in the earlier kindred of Xenacanthus. Diphyrcy as represented in *Ceratodus* has been (as far as the writer is aware) generally regarded as the most primitive condition of the body terminal of aquatic vertebrates.¹ McCoy¹⁹ in 1848 and Cope²⁰ in 1871, in their comments on

¹ Since the above was written, the memoir of W. N. Parker on *Protopterus* (Trans. Roy. Irish Acad., 1892) has been received. His view in this matter, strengthening an earlier influence of Dr. Traquair (Brit. Ass. Rep., 1871), is that "it is impossible to draw any conclusions with regard to the ancestral form of caudal fin in the Dipnoi from a study of adult forms."

diphycercy, have carefully reviewed this primitive condition from the standpoint of the palaeontological material then extant. Ryder²¹ in 1884, treating the subject of fish fins mainly from the embryological side, has given a most important memoir upon the evolution of the tail of fishes, comparing his results critically with those of Kölliker, Vogt, Dohrn and Lotz. He brings out clearly the degenerate stage in the development of the tail, whereby the homocercal is reduced to a diphycercal type which he terms *gephyrocercy*.

Reviewing the matter carefully, in view of the puzzling caudal structures of *Cladoselache*, the present writer is led to suggest that the origin of the caudal of gnathostome fishes is to be derived not through a diphycercal, but through a heterocercal condition. He believes, furthermore, that embryological results might in this way be interpreted. That phylogenetically the heterocercal type itself may have been evolved from some form of diphycercy, wanting, however, in radials, would appear extremely probable.

From the standpoint of palaeontology, there can certainly be little doubt that the heterocercal condition can easily claim priority in time, — heterocercy is represented in all Elasmobranchs (Xenacanthids excepted), in all known Ostracoderms. In recent forms it maintains in chondrosteian Teleostomes; either in adult or young, and in bony teleostomes as a regular embryonic condition. The only fish group whereby true diphycercy can claim antiquity among fossil forms is the Dipnoan, since the condition of caudal in Crossopterygians is generally heterocercal in the most ancient types, *Holoptychius*, *Osteolepis*.

If the fins of Xenacanthids are reduced to selachian conditions, why should not the tail, in view of its homologous origin, be regarded as also derivable from a selachian tail type? If dipnoan-like fins in this group are secondarily acquired, why should not also the dipnoan tail structures? If it is shown that the lateral fins of Xenacanthids may be reduced to the more ancient and more primitive type of *Cladoselache*, the tail structure in these forms, simple though it appears, might equally well be regarded as of acquired character.

Diphycercy in its existing conditions, with radials developed, as in *Ceratodus*, is, in the opinion of the present writer, a specialized, perhaps more strictly a degenerate condition, directly comparable with gephyrocercy, as shown in *Echiodon*, figured by Ryder.¹

On the side of embryology confirmation as to the antiquity of heterocercy is singularly clear, if the question of continuous dermal fold and of larval fin hair-rays be placed aside. Certain it is that the cartilage tail supports, radials, occur first on the ventral side, and have here increased to a remarkable size, often fusing, before the epural supports come to be formed.² The stimuli that give rise to this outgrowth of the caudal lobe have been closely followed by Ryder,³ whether or not we accept his views as to the exact manner of causation. That the tendency was from the earliest towards heterocercy is seen in the primitive outgrowth of the lower lobe of the tail, and in the consequent upturning of the fin end of the notochord. That epurally this axis became strengthened by variously grouped neural plates may clearly be seen in the embryos of flounder, salmon, shad, *Amia* or *Lepidosteus*.²² It is further noteworthy that the hypural border of the upper tail lobe tends for a long while to remain rayless, as is well seen in the young of *Lepidosteus* (or of flounder⁴). The free tip of the chord at a later stage is known to become regularly reduced, surrounded by growing and fusing radials or basals, or, in the case of *Chimaera*, curiously filamentous.⁵ It is, perhaps, significant that there seems in every case a stage in development when the heterocercal tail suggests a forking character, though this stage may be quickly outgrown and masked by a bending together of the lobes.⁶ In one of the stages in the development of the flounder⁷ would be represented the actual condition of *Cladoselache*, if

¹ Ref. 21, p. 1098, Pl. VIII, Fig. 3.

² Cf. l. c., Pls. I, II, III, IV and IX.

³ l. c., p. 1057.

⁴ Ref. 21, Pl. I, Fig. 7.

⁵ Balfour and Parker, Phil. Trans., Pl. II, 1882, p. 408.

⁶ Ref. 21, Pl. I, Figs. 7, 8, 9.

⁷ l. c., Pl. I, Fig. 7.

the tip of the chord be upturned, the (derm) rays absent, and a stouter outgrowth of the cartilaginous radials.

Among ancient forms all stages of gradation from heterocercy to diphyrcercy might well be illustrated in the ancient Crossopterygians, *Holoptychius*, *Osteolepis*, *Gyroptychius*, *Glyptolaemus*. Further degeneration in these forms might result in typical gephyrocercal types, whose transitional stages would be represented in Coelacanthids, *e.g.*, *Cocclacanthus*, *Undina*, *Diplurus*, *Macropoma*. From the tail type of *Cladoselache* diphyrcercy, as shown in *Xenacanthus*, might readily have taken its origin; it would have required merely the continuation posteriorly of the hypural rays and a gradual downturning or degeneration of the tip of the chord,—a condition specialized to environment, which among recent forms is clearly evolved in the case of the eel.

This view as to the derived character of what is accepted as diphyrcercy certainly assists not a little in closing phylogenetic ties. It adds further evidence for the nearing of Dipnoid as well as of Crossopterygian forms to the stem ancestral of Xenacanthids. It aids, moreover, in the comparison of Xenacanthids with more ancient and more generalized Elasmobranchs.

THE UNPAIRED FINS.

The structure of the unpaired fins of *Cladoselache* would naturally be expected to prove of great morphological interest. The present material, unfortunately, does not permit any satisfactory determinations. In a single specimen is preserved a detached fin of *Cladoselache kepleri* (?) which is entirely different in type from pectoral or ventral, and might, perhaps, be regarded as dorsal.

The specimen (Fig. 4) presents nine fin rays (radials) in a graded series, of which the foremost is the shortest. In this region, as in the other fins, the rays are concentrating and fusing, are most erect, perhaps may even have inclined slightly forward. The caudad rays are most inclined, abruptly tapering and become distinctly hollow in their basal half. It is interesting that each ray at its distal hinder edge has split off a bridge-

like bar which passes over and appears to be attached to its next neighbor, thus forming a cartilaginous margin to the fin web. The rays show a trace (?) of but a single basal support. It is evident that this material is in no way sufficient for generalizations, — *e.g.*, as to the beginnings of unpaired fins, or as to the troubled question of dorsal spines in *Cladoselache*.

One is, however, tempted to comment on the mode of strengthening the distal web margin, which *Polypterus* has so aptly specialized, in all probability as a neomorph. So, too, the foremost rays in their process of clustering might readily be adduced to prove the mode of origin of a dorsal spine. Or the hollow nature of the hindermost (and therefore the least modified) rays might be emphasized as typifying the most ancient form of fin support.

This specimen will become of value when others are found ; — in the present paper it is described as the only known remains of an unpaired fin.

THE SHAGREEN AND DERMAL DEFENCES.

Palaeozoic sharks seem as a rule to have been richly provided with dermal defences ; many and characteristic spines were evolved, specialized in their characters to a remarkable degree, as in the hook-like head spines of *Hybodus* and *Acrodus*, or in the fin spines of *Acanthodes* or *Diplacanthus* ; shagreen was often stout, tuberculate and sculptured, at times 'ganoid' in its outward characters, varying often in its coarseness of texture in different regions. *Xenacanthids*, as well as *Chondrenchelys*, however, appear to have been shagreened with the finest of denticles, a deficiency in dermal defences perhaps of a secondary nature, since in the former instance at least a median spine was present. In *Cladoselache*, it is to be noted that fineness of shagreen texture is unaccompanied by spines, — as far at least as can be positively judged from the present material. In this form the denticles become larger in size at the sides of the head, in the region of the jaw angle (suggestive, perhaps, of *Chlamydoselache*) and in a marked way on the anterior margin of fins and tail. The denticles are usually lozenge-shaped, varying in shape and

stoutness in different regions. Those that have fused together to form the circum-orbital plates have already been commented upon by Jaekel, whose figure, slightly modified by the writer, is here reproduced (Fig. 5). An entire ring of derm-plates as shown in a more perfectly preserved specimen (*C. fylei*) is also figured (Fig. 6). Their Acanthodian characters have been noted.

Dermal investiture of the fins, as already seen, is as yet specialized in the development of only the most minute rays. These appear to be intercalated between the primitive cartilage rays. In the pectorals they suggest from their extreme fineness of character, the slightest plaitings of superficial derm layer (Fig. 1). In the ventrals, however, they are not to be positively defined. Those of the tail are clearly marked, and have evidently been of a degree of usefulness consequent, perhaps, of the absence of cartilaginous supports in the upper lobe. They certainly agree in arrangement and direction with the firm dermal supports of the upper lobe in modern sharks. It is of especial interest that in the paired fins of this ancient form the superficial rays are intercalated, and not joined on to the endoskeletal part in a fringing line; it is thus suggested that the protrusion of these parts from between the cartilages, which gave them direction as well as support, was the origin of the dermal fin. Where greater rigidity combined with lightness was required, as in the outer posterior border of fin, the cartilaginous radials were obliged to resort to processes of forking, splitting and interwedging their pointed tips, devices which could not long remain in competition with the dermal ridges which now became acquired secondarily. The latter have, in paired fins, developed on the same lines as the older structure, have spread between them, and usurping their functions, have caused their degeneration.

Of lateral line nothing is determinable in specimens that are to be positively referred to the type genus. A portion of a shark from the locality in which *Cladoselache* was found shows clearly a lateral line, which is figured (Fig. 7), although but doubtfully referred to this form. It is especially interesting

that the lateral canal is an open one as in *Chlamydoselache*, and is bordered by stouter and more numerous denticles. The relation of their size to that of those of the surrounding body wall might be looked upon as significant of the primitive mode of encasement of the sensory tract.

THE DENTITION.

Comparison of a series of jaws has rendered it possible to understand the general characters of dentition. The head of *Cladoselache* viewed from below, even in its crushed condition, is strongly suggestive of *Chlamydoselache*, especially in the incurving, fringing teeth of the upper jaw. Like the modern form, its teeth were largest, longest and most acutely pointed symphysially, and shortest and smallest at the angle of the mouth. It has apparently the two anterior rows of the upper jaw separated by a depression, probably toothless, passing inward between. It is impossible as yet to say that there existed a symphyisial unpaired row of teeth on the lower jaw to be opposed to this depression. The broad horizontal bases of the teeth were not, however, arranged in fore and aft rows, with alley-ways between.

In *Cladoselache fylleri* each ramus of either jaw was closely studded with about twenty-five (ecto-entad) banks of teeth, each bank containing about eight teeth.

These ecto-entad banks, as already suggested, vary in general character in different regions of the mouth. Each tooth is wedged in between its neighbors of the right and left banks so that one lateral denticle becomes the buttress of the main cusp of its right neighbor, the other denticle often going no further than opposite the lateral denticle of its left neighbor. The general curve of the jaw gives an even and close-set appearance to the dentition. The symphyisial rows, though consisting of the largest teeth, have not been satisfactorily determined. Their outermost teeth possess an extremely long principal cusp, whose length is about one-ninth the distance from snout tip to articulation of jaw. These appear to have been greatly incurved, and were notably larger than their succeeding neighbors in the same bank. The lateral denticles were

not marked in the symphysial rows. The development of the lateral denticles, outer and inner, as we pass to the angle of the mouth, leads naturally to a condition that is strikingly hybodont. (Cf. *Synechodus*.) Hybodont, too, is the fact that (1) the marginal rows are the smaller and rounder and more degenerate in cusp characters, and (2) that the greatest wearing line appears to fall upon the middle members of each bank. The teeth typical of *Cladodus* occur in the rows of the anterior third of each jaw; here the length of the lateral denticles is about one-half that of the main cusp. Incurving of teeth is most marked in the front of the mouth, the direction of cusp becoming nearly parallel to its broad base. Underlying teeth appear to assume the *s*-like form. Teeth in the region of the mouth angle, become slightly unsymmetrical. Entire specimens, which represent perhaps young animals, show little abrasion of cusps. The teeth of an entire mouth of a large individual of *Cladodus* (?) *terrelli* indicate that wearing action had been greatest little more than midway from symphysis of jaw to articulation,—the pointed cusps, stout heavy ones in most cases, being ground away to their bases; and the appearance of the entire jaw is in consequence decidedly hybodont, none the less so as the teeth of jaw margin and mouth angle became small and bluntly chisel-like.

There appears to be present in *Cladoselache* no shagreen denticles at the outer margin of each jaw, which might be mistaken for teeth.

Eyes and nares are prominently marked in nearly all specimens. The olfactory capsules were terminal, large, and seem to have been placed quite closely together. The orbits were placed well forward in the head, the capsules appearing to be larger proportionally than those of *Chlamydoselache*.

Chlamydoselache may be looked upon as representing one type of Cladodont dentition. It does not agree with that of *Cladoselache* in a number of important characters. Its teeth, for example, are small, uniform in shape, decreasing in size from the middle line to mouth angle, and from the outer to the inner rows, each row with an alley-way separating it from its neighbor, each lateral denticle almost as serviceable as a

median cusp. In no instance, is the writer aware that the cusps have been found worn by usage. In *Cladoselache*, on the other hand, appears a character transitional to hybodont, — its teeth vary in a marked manner in size and shape. Except in the anterior portion of the mouth, the teeth are larger on the inner than on the outer mouth margin, the outer teeth are reduced in size, lose the prominent character of median cusp but augment in solidity and breadth. They secure solidity by interwedging their broad bases, but lose thereby the ingoing alley-ways; the lateral cusps function principally as neatly fitted buttresses to the central cusp of a succeeding neighbor. Even the longer cusps come in certain parts of the mouth to present a greatly worn appearance, suggestive of permanent character, as Dr. Newberry has already noted.

CONCLUSIONS.

Cladoselache in summary presents historic evidence as to the mode of origin of a number of shark structures. Its archaic characters, to be expected, perhaps, from its early occurrence, appear to allow no other interpretation than that of Wiedersheim as to the derived nature of Xenacanthid fins. It gives, moreover, evidence as to the antiquity of fins strikingly lateral-fold-like in character. As to this evidence, the conservative deductions of Jaekel, even had his observations been justified by the material recently discovered, seem to the present writer exceedingly debatable. Thus, for example, his conclusions as to the morphological value of the embryonic structures of shark and Torpedo seem by no means convincing. Admitting that in youngest stages the fin fold of a later derived form, Torpedo, may present a condition more archaic, it does not follow necessarily that in sharks there could not primitively have existed a continuous fin fold. On the other hand, in the present stage of our knowledge of the retarding and acceleration of embryonic structures, Jaekel's views would seem all the more questionable. Equally well might the living conditions of the later derived form have selectively evolved a latent ancestral character, which, reacquired, might

be emphasized by very early development. The significance of the heterocercal tail of *Cladoselache* is of especial importance, since this form occurs earlier than Xenacanthid. It suggests that the diphyrcery of existing gnathostomes might be secondarily acquired.

Cladoselache, in view of its generalized characters, might naturally be looked to for more accurate knowledge as to the phylogeny of the ancient Elasmobranchs. Fin and tail structure would appear to indicate that the ancestors of Xenacanthids may not have been widely separated from *Cladoselache*.

Especially interesting is the light that *Cladoselache* gives as to what may have been some of the characters of the Elasmobranch stem, from which the Diplacanth and Acanthodians may have had their origin. Comparison with Acanthodians brings out many points of agreement.¹ Shape and outward proportions were similar, gills were protected by frills of integument, eyes were similar in position and in their protecting ring of bony plates; feebleness in axial parts, and characters of dermal denticles were common to both; in lateral line a correspondence may, with probability, be traced; tail characters were at least similar; similar, too, in these forms appear the myocommata, whose clearly-marked character in fossils might indicate an unusual thickness or compactness of the connective tissue which separated the myomeres. Even the most bizarre characters of Acanthodian, lateral fin spines, are not altogether incomparable, especially when the evolution of the Acanthodian paired fins is taken into account as explained by Smith Woodward.² Thus in this fin the clustering of the unjointed radials to the anterior fin margin is not unsuggestive of fusion into a single plate, especially as it would appear that the lateral fins functioned as balancers, were incapable of great upward or downward motion. Fusion of the anterior radials might result in a sturdy spine, while degeneration and fusion of the posterior radials, which would leave a free fin web, might be the origin of the basal

¹ General affinities to Elasmobranchs have been summarized by Reis (Sitz.-Ber. d. Gesell. naturforsch. Freunde, Berlin, Nov. 15, 1892).

² Ref. 8, vol. II, p. 5.

cartilage figured by Smith Woodward¹ for *Acanthodes* and *Parexus*.²

Dentition, on the other hand, is to a noteworthy degree suggestive of Hybodont; teeth often appear worn; in lateral region tend to become broad and blunt; their grouping is in interwedging banks. In this regard *Cladoselache* differs from *Chlamydoselache*, whose frilling gill integuments seem the only striking point of comparison between modern and ancient form.

In conclusion a modification in the arrangement of the lowest sharks might be proposed. *Cladoselachids* would naturally be removed from the order *Ichthyotomi* (including *Pleuracanthids*), Cope. And, based on the extremely primitive fin girdle and tail characters, which *Cladoselache* and *Acanthodian* together present, the writer would suggest that these forms might be grouped together at the base of the *Elasmobranch* sub-class in an order which might be termed *Pleuropterygii*, in allusion to the fin axis not protruding from the body wall. In such a group *Cladoselachids* and *Acanthodians* would take subordinal rank.

This arrangement would have the merit of placing the *Acanthodian* group, which has proven difficult to adjust on account of its puzzling specializations, in a position where, from its appearance in time, one would naturally expect it, — near the base of the *Elasmobranch* stem, among more generalized forms.

Divisions might thus be defined: Subclass, *Elasmobranchii*;

ORDER I, PLEUROPTERYGII.

Notochord unconstricted; endoskeletal cartilages permeated with minute granular calcifications; (membrane bones sometimes occurring as in the specialized (*Acanthodian*) sub-order). Tail broadly heterocercal, lacking hypural supports in upper lobe; paired fins appearing as remnants of the primitive lateral fold, and functioning probably as balancers, directed somewhat downward; the line of basalia imbedded in the body-wall, its

¹ L. c., II, pp. 4, 35.

² The second pectoral fin spine in *Diplacanthus* might be explained on the ground of the approximation of the ventral spine next posterior. Cf. Cope, *Am. Nat.*, 1890, p. 413.

caudad end not protruding; radialia, with little trace of jointed structure, extending from body-wall to fin tip, tending to concentrate and fuse in the *anterior* fin margin.¹ Claspers absent. A circum-orbital ring of derm plates. Evidence of loose integumentary gill flaps. Myocommata often preserved in fossils.

SUBORDER I, CLADOSELACHII.

Membrane bones together with neural and haemal spines lacking. Suspensorium probably short and down-turned. In paired fins concrescence of anterior elements giving rise to specialization of radialia, and tending to rotate entad the fused basalia; the anterior fin region therefore becoming the more modified, tending to mask its structural characters: in pectoral the specialization of anterior radials producing a bow-like fin margin; in ventral the foremost basalia as yet unfused: the fins' body angle, anterior and posterior (horizontal), rounded by dermal investiture, the remnant perhaps of the continuous lateral fold. Circum-orbital ring of many derm plates.

FAMILY CLADOSELACHIDAE.

Body fusiform, presenting a horizontal dermal keel at the base of the tail. Fins bluntly lobate: in pectoral the posterior radials often bifurcate. Teeth cladodont. Anal and dorsal probably small, lacking in fin spine (?). Circum-orbital derm plates quadrangular and in concentric rows.

GENUS CLADOSELACHE.

Body bluntly fusiform. Teeth cladodont, p. 106. Shagreen varying in body regions. In pectoral delicate (dermal) rays between radialia, radialia in character as described p. 92. Tail, with basal supports arranged as shown p. 98, exhibiting fine (dermal) ray structure in place of hypurals of upper lobe.

The Acanthodians would then follow as presented by Smith Woodward, but regarded as a sub-order.

¹ In the order Selachii on the other hand, the concentration, splitting and fusing of the radialia is at the posterior (distal) fin end, consequent of the protrusion of the tip of the basalia in this region.

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EXPLANATION OF FIGURES.

FIG. 1. Pectoral fin. $\times \frac{1}{5}$. *Ax.* Axil of fin. *Bas.* Basalia. *BW.* Body wall. *D.* Dermal fin-margin. *Rad.* Radialia. *T.* Trichinost.

FIG. 2. Ventral fin. $\times 1$. *Bas.* Basalia. *BW.* Body wall. *D.* Dermal fin-margin. *Rad.* Radialia.

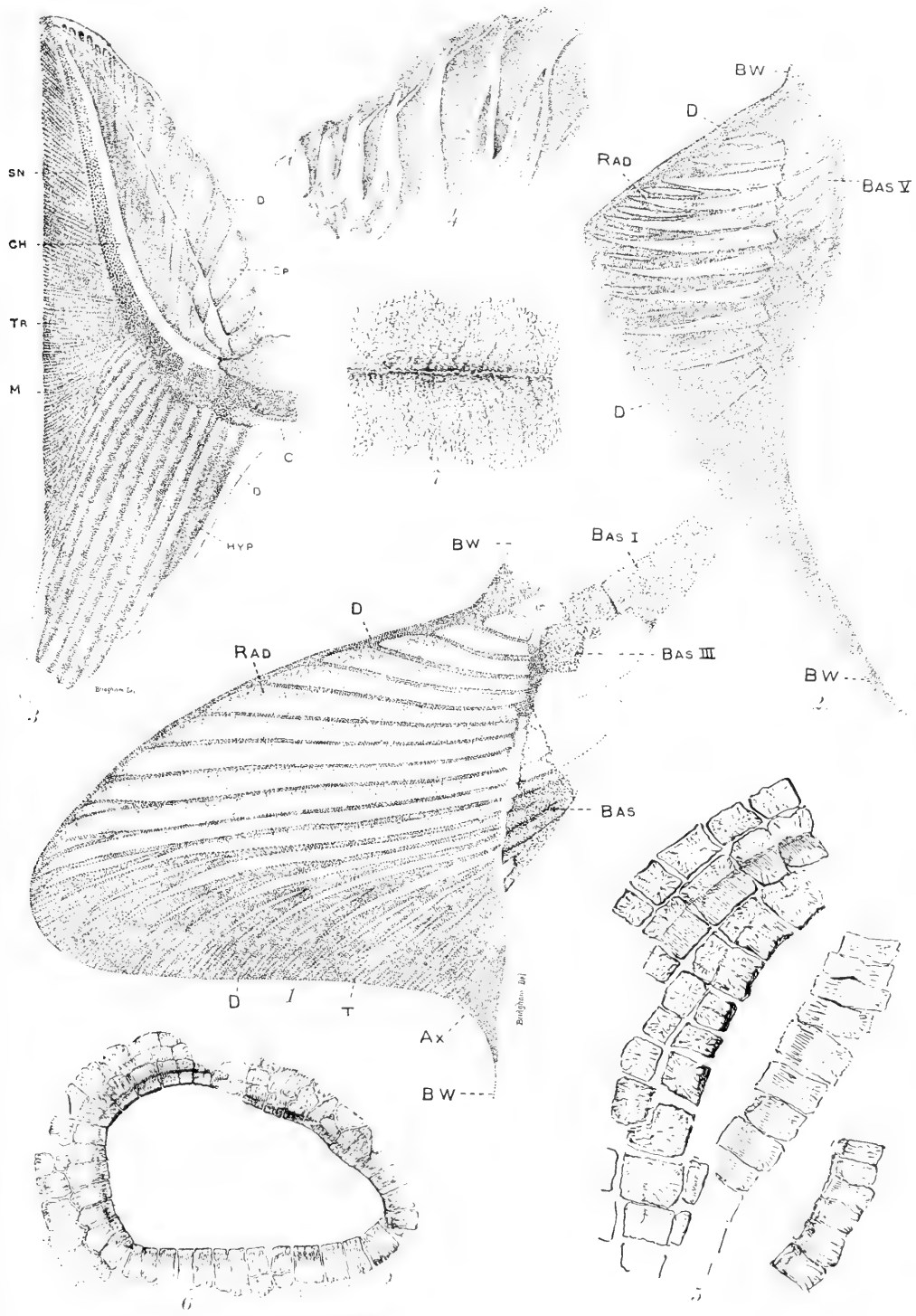
FIG. 3. Tail. $\times \frac{2}{3}$. *C.* Lateral view of horizontal cut-water. *CH.* Notochord. *D.* Dermal fin-margin. *Ep.* Epural plates. *Hyp.* Hypural rods. *M.* Membranous hypural margin of upper lobe. *SN.* Sub-notochordal rod. *Tr.* Trichinosts.

FIG. 4. Unpaired fin (dorsal?). $\times 1$. Showing conical radial cartilages, whose distal ends split posteriorly to form marginal rim of fin web.

FIG. 5. Circum-orbital derm plates. After Jaekel (drawing slightly modified from fin type-specimen).

FIG. 6. Circum-orbital ring of derm plates ($\times 1$).

FIG. 7. Lateral line ($\times 1$). The arrangement of the dermal denticles indicating the presence of an open groove as in *Chlamydoselache*.



THE OPTIC VESICLES OF ELASMOBRANCHS AND THEIR SERIAL RELATION TO OTHER STRUCT- URES ON THE CEPHALIC PLATE.

WILLIAM A. LOCY.

THE evidence is increasing that the different organs of special sensation in vertebrates all belong to one series and are modifications of a sensory basis of common origin.

The derivation of the ears from the organs of the lateral line system, seems now to be established ;¹ but the relationship of the eyes is still obscure, and they are not commonly admitted to the same group with the ears and other serial sense organs. The fact that the eyes spring from epithelium of the neural plate, while the other serial sense organs have, presumably, an independent epiblastic origin, is regarded by many as sufficient evidence that the eyes belong to a different series and cannot be identified with the other sense organs. At the present time, I think most morphologists would not feel warranted in going further than to assume the possibility of the eyes belonging to the same class with the other ganglionic sense organs.

Froriep,² in 1885, published the researches from which the modern views on the relation of sense organs take their departure, and Beard³ almost simultaneously published the results of his studies on the sense organs, giving the name "Branchial sense organ" to certain ones located just above the branchial region. Beard, in another paper, is especially strenuous against admitting the eyes into the same category with the ears and organs of the lateral line system.

The writers who have since touched upon the question, opened up by the researches of Froriep and Beard, are very

¹ Ayers, A Contribution to the Morphology of the Vertebrate Ear, etc., *Journ. Morph.*, Vol. 6, Nos. 1 and 2, May, 1892.

² Froriep, *Hist. Archiv.*, 1885.

³ Beard, *Quar. Journ. Mic. Sci.*, November, 1885.

numerous and cannot be severally referred to in the present article.

Whitman¹ has placed all the special sense organs in one group, expressing the conviction "that the segmental organs of annelids have formed the starting point for the development of the organs of special sense in the higher animals, not excepting even the eyes of vertebrates." It is not, however, his suggestion of invertebrate relationships that I wish to bring out at the present moment, but the fact that he places the eye in a common group with the other sense organs. Contrary views are still held by many anatomists, and the whole question of the affinities of the vertebrate eye is in such shape, that any contribution which may throw light on the subject will be of interest to Morphologists. It is for that reason that I have been led to offer, as a preliminary communication, some recently made observations of my own on the early development of the eyes, and other sensory organs, which arise in a similar manner, on the cephalic plate of elasmobranch embryos.

I have shown in a previous number of this JOURNAL² that certain steps in the early formation of elasmobranch embryos have escaped the attention of previous observers, and the first steps in the formation of the eye, also take place in the same stages of development that have been hitherto insufficiently studied. The elasmobranchs are especially favorable for studying the formation of the primary optic vesicles, but, strangely enough, their early history in these animals has been completely overlooked.

Balfour³ said, "The eye does not present in its early development any very especial features of interest" and this statement seems to have withdrawn attention from that organ in elasmobranch fishes. The Zieglers,⁴ whose memoir on the

¹ Whitman, Some New Facts about the Hirudinea, *Journ. Morph.*, Vol. 2, No. 3, April, 1889.

² Locy, The Formation of the Medullary Groove, and some other Features of Embryonic Development in the Elasmobranchs, *Journ. Morph.*, Vol. 8, No. 2, May, 1893.

³ Balfour, Monograph on the Development of the Elasmobranch Fishes, p. 184.

⁴ Ziegler, H. E. & F., Beiträge zur Entwicklungsgeschichte von Torpedo, *Archiv für Mik. Anat.*, Bd. 39, Heft 1, January, 1892.

early stages of development of Torpedo was published as recently as last year, failed to note the optic vesicles which are so well developed (in Acanthias at least) at the stage which has been designated by them 'Stage D.' As I shall presently show, the optic vesicles appear at a considerably earlier period. I have examined Professor Ziegler's figures, and, also, the models he has made from them, but I do not find traces in either, of the optic vesicles nor of the other related sensory depressions of which I shall soon speak. The Zieglers worked upon Torpedo, and my own observations were made upon *Squalus acanthias*, Linn., of the western Atlantic coast.

The involutions that are to give rise to the optic vesicles appear at a very early stage. As soon as the cephalic plate has been formed, by an expansion of the anterior end of the embryo, two faint circular depressions are to be seen upon its extreme anterior surface (Fig. 1, *op.*). These depressions grow deeper, and run together in the middle line, and the continuous infolding produced in this way divides the cephalic plate into an anterior depressed region, and a posterior elevated region.

The infolding gives rise, also, to the infundibulum.

The optic vesicles, which are started near the median line, grow outwards laterally, and come to occupy the lateral parts of the depressed region, but as the infolding forming it does not extend completely across the cephalic plate, the optic vesicles do not reach the margin of the medullary folds. The latter are broadly expanded beyond the optic vesicles (Figs. 2 and 3).

When distinctly formed, the optic vesicles are circular in outline, concave from within, and they form rounded elevations on the outside (Figs. 3 and 4, *op.*) where the cups come in contact with the outer layer of the epiblast.

The earliest stage in which I have noted the circular areas shows three mesoblastic somites, and the medullary folds of both head and body are not only broadly open, but they are even ventrally curved. It is a stage occurring intermediate between the stages designated *C* and *D*, respectively, by the

Zieglers, but it is a distinct stage which is not figured nor described by them.

This early appearance of the basis of the eye, before the formation of the medullary canal, has for a long time been known to occur in the mammals, but it was regarded as precocious development in that class of animals. It has been described and figured in the mole, by Heape,¹ and, more recently has been noted in Birds by Duval and other observers, in Necturus, by Whitman,² and in Necturus and other amphibia by Eycleshymer.³ So far as I am aware the early condition in Elasmobranchs was described for the first time in my paper⁴ before referred to.

The optic vesicles are clearly outlined in *Squalus acanthias*, before the medullary groove is fairly established, and long before the medullary canal is formed in any part of the embryo.

But, more interesting than the fact of their very early appearance in Elasmobranchs, is their apparent relationship to other depressions that are formed upon the cephalic plate behind the already established optic vesicles.

The new involutions referred to make their appearance upon the cephalic plate just back of the optic vesicles. Two of them (Figs. 3 and 5^{1,2}) take precedence of all others in development, and they are so distinctly formed as to afford a good basis for comparison with the optic vesicles. They are circular depressions formed in precisely the same manner as the optic vesicles in front of them, and they produce upon the exterior corresponding rounded elevations. The optic vesicles are formed first, and when, at a very little later stage, the others arise behind them, it appears as if the process of eye formation were repeating itself serially.

The anterior one is larger, and makes the nearest approach to the eye vesicle in size and structure; the second is smaller, and is faintly bilobed.

In Fig. 3, these structures are shown as they first appear, and in Figs. 4 and 5, they are shown, both from within and

¹ Heape, *Quar. Journ. Mic. Sci.*, Vol. 8, Oct., 1886.

² *Journ. Morph.*, Vol. 2, April, 1889.

³ *Journ. Morph.*, Vol. 8, March, 1893.

⁴ *Journ. Morph.*, Vol. 8, May, 1893.

without, after they have become distinctly circular in outline and cup-like in character.

These circular pockets not only arise in a similar way, but structurally resemble the optic vesicles. In cross and longitudinal sections, the cells of the sunken patches are similar to those in the eye pockets. They may be properly designated *accessory optic vesicles*.

Other circular depressions arise later, upon the cephalic plate, behind the two just described, but they are less obvious

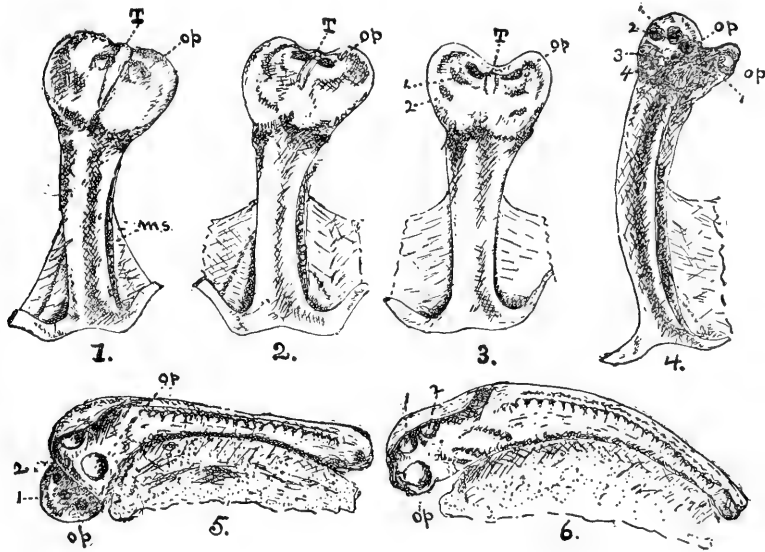


FIG. 1.—Embryo with 3 mesoblastic somites, just after formation of the cephalic plate, showing beginning of optic vesicles.

FIG. 2.—Embryo with 9 somites, showing optic vesicle established.

FIG. 3.—Somewhat older embryo, showing optic vesicles, and the first appearance of the two homologous depressions.

FIG. 4.—Embryo with 17 somites, after the rise of the medullary folds, showing the optic vesicles, the two homologous depressions, and two supplementary depressions.

FIG. 5.—Slightly older embryo, placed so as to give a view into the front of the medullary groove.

FIG. 6.—Embryo of about the same age, seen from the side, showing (1, 2) structures homologous with the optic vesicle.

All the figures are from photographs \times about 20 diameters.

T, central tongue-like elevation on cephalic plate; op., optic vesicles; 1, 2, depressions homologous with the optic vesicles; 3, 4, depressions appearing later, possibly homologous with 1 and 2.

and are obscured by neuromeres arising in the same region. I think that at least two of them (Fig. 4, 3, 4) should be classed with the former, but upon that point, I should prefer to reserve my judgment. I shall publish later a more detailed description of this region of the cephalic plate with figures of sections.

If the view here expressed is true, we have a multiple-eyed condition in the embryos of these animals—a condition common enough in adult invertebrates, but not known to persist in vertebrated animals,—and this takes us one step towards the ancestral condition.

While these structures have been forming, the medullary folds of the head have been growing upwards, rising more rapidly in the anterior part of the cephalic plate, around the optic vesicles, and more slowly further back. Figs. 4 and 5 show embryos after the medullary folds have begun to grow upwards. In Fig. 5, the optic elevation (*op.*) is seen from the outside, and also the two elevations (*1*, *2*) corresponding to the first and second circular depressions behind the eye; the others are hidden from view in this figure by a flexure of the medullary fold, which, by the way, is a normal condition at this stage.

For a brief time after the medullary folds meet in the median line, the external elevations are all visible, but that region of the head, behind the optic vesicles and in front of the ears, becomes the seat of great modifications, and the structures described give way to later formed ones. They are, therefore, not only embryonic structures, but they are also transitory in nature. It is a truism in development, that the more primitive characteristics appear first, and the secondary modifications come in later. The structures described are, then, among the most primitive that have been preserved in this group of fishes, and should be of significance in indicating the ancestral relations of the eye.

It has been urged as an insurmountable objection by Beard and others that the eye cannot be homologized with any sense organ developed outside the neural plate. But the suggestion that the neural plate is undoubtedly very much widened and

expanded from its ancestral condition, and that certain sensory areas, originally lying outside, may have been included in it, does much to offset that objection. It is not improbable that there is neural plate material in the ear and other sensory organs of its same rank.

Kupffer,¹ has shown in *Petromyzon*, that the ganglionic elements of the head region make two distinct rows of fusions with the epiblast. The upper set corresponds to the ganglionic sense organs, and the lateral line of comparative anatomy, and the lower, or epibranchial set, corresponds to the organs described by Beard under the name of Branchial sense organs.

The observations I have recorded above afford evidence that the lateral eyes are derived from segmental organs — segmental in the sense that they occur in pairs serially arranged. Three pairs of these organs are clearly defined on the cephalic plate. The front pair is the optic vesicles, and the other two pairs I have called accessory optic vesicles.

This condition implies an ancestral form possessing serially arranged eye-like sense organs.

The significant question arises here : What is the relation of this segmental series containing the eye as its highest differentiation, to the lateral line series, including the ear as the example of its highest development? Although we have not sufficient data to return an entirely satisfactory answer, nevertheless, the assumption that they are closely related is not without foundation. There certainly is no insurmountable objection to placing the eye and ear in parallel homologous series, one of which has become included in the expanded neural plate and the other has not. It is not even necessary to assume the independence of the two series. I think there is, to-day, really more evidence to support the view that they are genetically related than that they are entirely distinct from one another.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLL, MASS., July, 1893.

¹ Kupffer, *Verh. Anat. Ges.*, München, 1891.

NOTE. — Since the above article left my hands I have returned to the study of the structures described with a large collection of Elasmobranch embryos embracing stages which exhibit the early condition of the accessory optic vesicles, and also stages that show their later transformations. I have confirmed my observations previously made, and, further, I have been able to trace with certainty the anterior pair of accessory vesicles into the pineal outgrowth. It is therefore necessary to modify my statement above, that the structures are all transitory. It thus appears from my later observations that the pineal outgrowth arises from two pairs of vesicles that are homologous with those giving rise to the lateral eyes.

LAKE FOREST UNIVERSITY,
November 2, 1893.

PRELIMINARY OBSERVATIONS ON SOME CHANGES CAUSED IN THE NERVOUS TISSUES BY REAGENTS COMMONLY EMPLOYED TO HARDEN THEM.

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Introduction. — In determining pathological changes in animal tissues, the normal histological appearances are used for the purposes of comparison. These appearances, it is presumed, are to be found in animals suddenly killed while in full health.

As a matter of fact, the tissues obtained from such animals are not exactly similar, and it is now known that there are a number of secondary conditions which must be taken into account.

The normal tissues certainly vary, according to the age of the animal, and the physiological conditions of rest and fatigue just preceding death.

Probably this does not exhaust the list, but these two points are the only ones thus far demonstrated.

The methods of treating tissues for the purpose of histological examination are admitted by all investigators to produce more or less marked variations when compared with fresh material.

By these methods both the relative and absolute size of the different elements and their reactions to staining fluids and other reagents are modified.

All this is true, quite apart from the many changes which may be induced by careless or incautious handling.

One of the most disturbing reagents is the hardening fluid, and it is to some of the effects of hardening fluids that we wish in this paper to direct attention.

The study of this matter has two objects in view : first, to determine what changes are induced by the hardening reagents

generally employed; and second, in the light of these facts to discover, perhaps, some method which will leave the tissues more nearly normal than do those which are now employed. It is to the former object alone that we shall here attend.

The tissue which we have chosen for the subject of experiment is that forming the central nervous system of the vertebrates, and the chief observations have been directed to the change in the weight and volume and to the variations in solids and fluids while undergoing the hardening process.

The extent and importance of these changes were brought to our attention several years since, when the brain of Laura Bridgman was put into our hands for examination.

The brain had not been weighed upon its removal from the body, and it was thought desirable, if possible, to determine its weight when fresh.

This brain had been subjected for 45 days to Müller's fluid to which had been added $\frac{1}{6}$ its volume of 95% alcohol, and then to a solution of $2\frac{1}{2}\%$ bichromate of potash for 44 days more, before it was tested with a view to obtaining its fresh weight.

Control experiments, made upon other brains, indicated a very great increase in weight as the result of a treatment nearly similar to that to which the Bridgman brain had been subjected. Following this hint, a somewhat more extensive series of observations was undertaken to determine the conditions controlling this increase in weight, and these observations furnish the material for this present paper. The standpoint from which they have been made has been rather chemical and physical than histological, and we have not prepared specimens for the microscope from material hardened in different ways, because, until the number of methods of hardening shall have been reduced to a minimum by means of some preliminary work, this latter point cannot be advantageously taken up.

History of Investigation.—The investigation was begun in January, 1890, at Clark University, Worcester, Mass. In connection with it, that Institution granted an appropriation for the study of the nervous tissues in man, and during the month of September, 1890, through the courtesy of Dr. Ferguson, the

work was carried on in the Pathological Laboratory of the New York Hospital. During that time we were indebted to a number of physicians in New York for material placed at our disposal.

During the winter of 1890-91, Dr. J. C. Cardwell and Prof. C. F. Hodge both took an active share in the observations; and the former made several independent series of tests which he kindly permits us to use. Since October, 1892, the work has been carried on in the Neurological Laboratory of the University of Chicago, and in its completion Mr. A. C. Eycleshymer has taken part.

Outline of Observations.—Our notes at date enable us to speak in some detail concerning the effects of the solutions of bichromate of potash and of alcohol on the weight, the volume and percentage of solids in the brain of the sheep, and in a more general way, concerning the effects of these and some other solutions on the brains of sharks and men as well.

SECTION I. — *Sheep's Brains.*

The results obtained can perhaps be most simply presented by following the history in a typical case and then noting the effects of varying the conditions which are there imposed.

Typical Case.—If the entire encephalon of a sheep is removed from the cranium some six hours after death, the animal having been killed by bleeding, it will be found that it can be taken out with comparative ease, if the hypophysis and the gray caps of the olfactory bulbs are neglected. This was the case in the brain of which we speak. The pia was always left on the brain.

After removal, the brain was cut longitudinally, thus giving two symmetrical halves (hemiencephala) approximately equal in weight.

The first experiments were made on hemiencephala of the sheep. Since each half of the encephalon contained the same proportion of the different encephalic structures, it was possible by this means to make two tests with the brain of one sheep.

The hemiencephalon was suspended by a thread in the middle of a jar of hardening fluid, and the jar was closed with a cover.

In the case of the bichromate of potash solution, the hardening was, as a rule, conducted in the dark, and at the ordinary temperature of the room, *i. e.*, 18–22° C.

The solutions of bichromate of potash were all made by adding the salt to the full measure of water.

For example, a 2% solution represented 2 grms. of the salt added to 100 c.c. of water.

The instance with which we shall begin was the following:—

January 16, 1890. The hemiencephalon of a sheep was found to weigh 59.8 grms.

This was placed, in the manner above described, in a jar containing 500 c.c. of 2½% solution of bichromate of potash in tap water. The specimen was then weighed at the intervals indicated in the subjoined table.

TABLE I.

Showing the change in the weight of the hemiencephalon of a sheep when subjected to the action of 2½% solution of bichromate of potash for 685 days:—

TIME IN DAYS.	PERCENTAGE GAIN.
1.0	17.8
2.2	26.2
3.9	31.4
5.9	34.2
12.9	36.2
03.0	37.1
550.0	37.1
638.0	39.0
685.0	38.0

The increase in weight which was found is given in the column of percentages, these percentages being based on the weight of the fresh specimen as noted above.

The examination of Table I shows that this specimen increased greatly in weight—that this increase was very rapid during the first day (24 hours), still active during the next five days, amounting at the end of the sixth day to

34.2% ; while during the next 679 days it increased less than 4%.

At the end of this time the specimen was well hardened, and the consistency was good ; it was, however, a trifle brittle.

The change in weight was determined by weighing the specimen from time to time, and the following were the conditions controlling the operation :—

The balances ordinarily used responded to 0.01 grm. At times more delicate balances were employed.

The specimen, as stated, was suspended in the fluid by means of a thread ; this thread when dry, weighed, with little variation, .05 grm. and gained almost 60% in weight after soaking in water or bichromate. The average hemiencephalon weighed when fresh about 56 grms., so that the weight of the thread would amount to less than 0.1% of this, which is insignificant for our purposes.

Since, at each weighing, the specimen had to be removed from the fluid and then drained for a time, it became of interest to observe how rapidly it lost weight by drainage, and how far this operation could safely be carried.

The principal conditions influencing the loss of weight by drainage are, of course, the time, the fluid and the temperature.

This last was practically the room-temperature, and varied somewhat with the season, being in winter about 18–22° C. The specimens were put in a funnel and allowed to drain usually for seven minutes before weighing. To show the effect of drainage the following Table 2 is given. The specimens were removed and allowed to drip one minute before weighing at all ; at the end of one minute the first weighing was made. They were then weighed at 2, 4, 6 and 8 minutes after the first weighing and the percentage loss in weight calculated from the weight taken after one minute, as a basis.

It will be noticed here that we give the percentage loss of specimens hardened in alcohol as well as those hardened in bichromate.

The fuller observations on alcohol are stated farther along.

TABLE 2.

Showing the percentage loss in weight by drainage. Specimens from 2% bichromate of potash :—

AFTER DRAINING. MINUTES.	SPEC. 2.	SPEC. 2.	SPEC. 3.	SPEC. 3.	SPEC. 4.	SPEC. 5.	AVERAGE.
2	0.9	0.8	0.36	0.9	0.60	0.5	0.66
4	1.3	1.3	0.53	1.4	0.79	0.8	1.02
6	1.5	1.5	0.77	1.6	1.2	0.9	1.24
8	1.6	1.7	0.8	1.7	1.3	1.0	1.35

Specimens from 94.5% alcohol :—

AFTER DRAINING. MINUTES.	SPEC. 2.	SPEC. 4.	SPEC. 1.	SPEC. 3.	AVERAGE.
2	0.9	0.6	0.8	0.8	0.77
4	1.3	1.0	1.0	1.1	1.1
6	1.6	1.4	1.4	1.6	1.5
8	1.9	1.4	1.6	1.8	1.6

These figures also show how similar the two weighings of the same specimen can be. The error due to weighing is therefore slight.

Extent of Drainage.—In this connection it must be remembered that drainage cannot be carried on very far without permanently altering the weight of the specimen.

The following figures show the loss in specimens already drained for seven minutes, and then left somewhat exposed :

A hemiencephalon hardened for 34 days in a 2½% solution of bichromate of potash was drained seven minutes, after which it weighed 82.3 grms. It was then wrapped in a thin layer of cotton saturated with the same solution, and the whole covered with paraffin paper. After 24 hours under these conditions it weighed 80.7 grms., thus having lost 2%. On being left for the next five days in the original fluid, it weighed 81.6 grms., thus showing a permanent loss of 0.9%.

This loss was not made good later. A second specimen hardened in a 2½% solution of bichromate of potash, plus ⅙ its volume of 95% alcohol showed, after similar treatment,

an initial loss of 2.3%, and a permanent loss of 1.3%. The third specimen hardened in 93% alcohol showed after similar treatment an initial loss of 2%, which proved permanent.

It appears from these observations that a comparatively small loss of fluid from the specimen may cause a permanent diminution in weight, and that drainage must not be continued for too long a time.

Modifying Conditions.—In pursuance of our plan we may now consider a number of questions concerning possible changes in the specimen, Table 1, under slightly different conditions. We shall first indicate those conditions which have been varied and found to be insignificant.

The combination of tap water or distilled water with either common bichromate of potash or that chemically pure (C. P.) does not influence the result.

The point was tested on 6 brains, giving 12 specimens.

Each of these was put in 800 c.c. of 2% bichromate of potash in the dark for 60 days. The specimens were all removed from the skull and weighed within two hours. The results are exhibited in Table 3, in the order of the removal of the brains, — the two halves of the same brain being indicated by a serial number and its prime.

TABLE 3.

SERIAL NUMBER.	BICHROMATE OF POTASH, 2 PER CENT.	WATER.	PERCENTAGE OF INCREASE IN WEIGHT.	DIFFERENCE BETWEEN HALVES.
15	Common.	Tap.	31.2	—
15'	C. P.	"	32.6	1.4
16	Common.	"	36.5	0.5
16'	C. P.	"	36.0	—
17	Common.	Distilled.	33.6	0.8
17'	C. P.	"	32.8	—
18	C. P.	"	36.5	—
18'	Common.	"	36.9	0.4
19	C. P.	Tap.	35.1	—
19'	Common.	Distilled.	35.6	0.5

It is evident from this table, that although treated in slightly different ways, the two halves of the same brain react in a similar manner. The comparison between the two halves of the same encephalon is much more reliable than between two encephala, as we shall see, so that a study of the small differences between specimens in different mixtures justifies the conclusion that neither chemically pure bichromate of potash nor distilled water effect any significant variations in the increase in weight.

Amount of Air, etc.—It was further determined that the amount of air enclosed in the jar holding the specimen was without significance.

The substance extractable by 2% bichromate of potash from the small cork sometimes used to suspend the specimen in the jar has no influence on the weight, nor did the point in the fluid column at which the specimen was suspended, affect the result, that is, the specimen reacted in the same way whether it was hung near the top of the jar or near the bottom.

The variations due to the quantity of fluid employed are slight, and appear to be easily explicable.

A series of four encephala were taken for this test. In each case one hemiencephalon was put into a small quantity of a 2% solution of bichromate of potash and the other into a large quantity, in which they remained for 27 days. As in Table 3, the number and its prime indicate the two halves of the same brain.

The following table shows the results obtained:—

TABLE 4.

SERIAL NUMBER.	QUANTITY OF FLUID.	FRESH WEIGHT.	PERCENTAGE GAIN AFTER 27 DAYS.
6	200 c.c.	49.71 grms.	41.0
6'	2500 "	49.61 "	41.1
8	200 "	45.00 "	35.6
8'	2500 "	49.00 "	34.7
10	400 "	47.69 "	37.0
10'	2500 "	50.72 "	35.2
12	400 "	47.48 "	33.1
12'	2500 "	47.50 "	32.4

Under these conditions, the two halves of the several brains increase in weight in about the same way, although here again there are marked differences between the several encephala employed. It is noticeable that the hemiencephalon in the smaller quantity of fluid has in the three instances out of the four, undergone the greater increase in weight.

This is the reaction to be expected for the following reasons : As we shall later see, the smaller the percentage of bichromate in the solution, the greater the increase in the weight of the specimen. As already stated, the average weight of a fresh hemiencephalon is 56 grms., and of this same 44.5 grms. are water, giving about 44 c.c. of water.

Sooner or later this water becomes mixed with the surrounding fluid, and when only 200 c.c. of a 2% solution of bichromate of potash are originally used, this addition of about 22% serves to reduce the percentage of the bichromate of potash to 1.7%, and since the solution containing the smaller percentage causes the greater increase in weight, we should expect the specimen in the small quantities of the solution to become the heavier, because the dilution effected by the tissue fluids is more marked in these cases.

Viewed in the light of this result, the renewal of the bichromate of potash solution in which the specimen is being hardened, should slightly retard the increase in its weight, because the new fluid would have a slightly larger proportion of the salt than that which had been diluted by the fluids of the specimen.

The variation due to the change of fluid, was, in the case of the sheep's brain, so slight that it was easily masked by other factors, and the expected influence of change was therefore not demonstrated.

The renewal of the fluid also prevents the formation of mould. Concerning the influence of such growths on the change in the weight of the specimen, we have no observations.

Light. — Variations in the amount of light were not found to influence the increase of weight. We turn next to the influence of the temperature. Six encephala were employed,

the hemiencephala from the same animal being kept under similar conditions.

Each hemiencephalon was put in 400 c.c. of the 2% bichromate of potash solution and allowed to harden. The temperature conditions are given in Table 5.

TABLE 5.

TEMPERATURE.		PERCENTAGE INCREASE IN WEIGHT.			
		AFTER 5 DAYS.	AFTER 12 DAYS.	AFTER 36 DAYS.	AFTER 54 DAYS.
38° C.	1	24.5	22.1	21.2	21.2 ¹
	1'	22.6	21.2	20.2	21.0
	2	24.9	23.4	22.4	22.0
	2'	25.2	22.6	20.9	23.3
		24.3	22.3	21.2	21.9
17° C.	3	27.5	32.1	32.9	32.5
	3'	26.8	30.9	30.4	30.3
	4	28.8	34.2	36.0	34.8
	4'	30.2	33.8	36.2	35.1
		28.3	32.7	33.9	33.1
10° C.	5	27.0	33.0	35.7	28.1 ²
	5'	25.3	31.2	33.5	32.4
	6	25.7	32.5	35.6	33.4
	6'	26.4	30.7	32.2	32.2
		26.1	31.8	34.2	32.6

The examination of Table 5 shows that at the end of 54 days the specimens at 38° C. have gained less in weight than the other groups ; that the groups at 17° C. and at 10° C. act practically in the same way, since no significance is to be attached to the differences between them. It is to be noted that there is a slight decrease in weight in the two groups last mentioned between the 36th and the 54th days. We shall

¹ At the end of 36 days, Specimen 1 was continued for 18 days at 10° C.

² Specimen 5 was in the same way continued for 18 days at 38° C.

meet this decrease following the attainment of the maximum, later on.

At the high temperature of 38° C. the increase in weight is not so great, and the subsequent decrease much greater than at the lower temperatures. Transferring the specimen after 36 days at 38° C. to 10° C. does not affect the weight (*vide* Specimen 1).

The reverse change (*vide* Specimen 5) causes a rapid and large loss in weight. At the same time there was a corresponding decrease in volume. The effect of high temperatures will need further special study, but the temperatures between 17° C. and 10° C. are without decided influence on the gain in weight.

Percentage of Salts.—It appears that within the limits employed, the greater the percentage of bichromate of potash the less the increase in weight. In support of this statement the following figures are given :—

						AVERAGE INCREASE IN WEIGHT.
4	Hemiencephala	after 20 days in	$\frac{1}{2}\%$	Bichromate Solution	=	59%
4	"	" " "	2%	" "	=	36%
2	"	" " "	4%	" "	=	24%
2	"	" " "	8%	" "	=	15%

There is, however, a difference in the reactions of specimens which these figures do not bring out, namely, that the smaller the percentage of bichromate of potash the more rapidly is the maximum weight attained. While the specimens in 8% bichromate of potash at the end of 640 days had increased from the figures above given (15%) to 21%, the specimen in 4% solution showed an increase to 30% at the end of the same time.

The relative differences in weight are slightly less at the end of this longer period, but the general character and significance of the table is not thereby affected.

Cause of Increase in Weight.—The increase in weight, under the action of the bichromate of potash is due to the absorption of both water and the salt by the specimen. This is shown by putting the specimen in a weighed quantity of fluid and determining that the increase in the weight of the specimen is equal to the decrease in the weight of the fluid.

Conversely, if the increase in weight is due to the absorption of the solution, the amount of solids in the hardened specimen should be equal to that in the fresh specimen plus the salts dissolved in the amount of the hardening fluid which has been absorbed.

Percentages of Water and Solids in the fresh Hemiencephalon of Sheep. — The average of ten specimens dried to a constant weight at 95° C. gives the solids in the sheep's brain as 20.5% of the fresh weight. The variation is from 19.4% to 21.2%, or 1.8%.

Thus far it has not been possible to bring the variations into relation with any other variable.

A similar determination was made for encephala which had been acted upon by 2% bichromate of potash for from 45 to 57 days.

The average percentage of solids was found to be 20.9% of the fresh weight for those hardened 45 days, and 21.9% for those hardened for 57 days.

TABLE 6.

FRESH WEIGHT OF HEMIENCEPHALON IN GRMS.	PERCENTAGE OF SOLIDS, FRESH BRAIN.	FRESH WEIGHT OF HEMIENCEPHALON IN GRMS.	PERCENTAGE OF SOLIDS AFTER 2 PER CENT. BICHROMATE OF POTASH.	
			FOR 45 DAYS.	FOR 57 DAYS.
60.4	20.0	50.7	21.9	
59.0	21.2	49.6	20.8	
55.8	21.2	49.0	20.5	
52.9	20.6	47.5	20.3	
52.9	20.7	60.3		21.4
52.9	20.2	60.2		22.1
52.5	20.6	59.4		22.0
51.7	19.4	54.5		21.7
48.6	20.1	51.2		22.4
46.0	21.1			
Average = 20.51		After 45 days = 20.9		After 57 days = 21.9

Since the variation is about the same in amount in both the fresh and hardened specimens, and the average is raised 0.4% and 1.4% respectively in the hardened specimens as compared

with the fresh ones, it is to be concluded that this process of hardening adds slightly to the total solids. This amount 0.4% and 1.4% of the total fresh weight is equal to 0.21 and 0.75 grms. The average quantity of fluid in the hardened specimens is about 50 c.c., which would contain nearly 1 gram. of the salt.

To the solids found, the percentage of bichromate of potash in the solution bears the same relation.

After 60 days in a solution of $\frac{1}{2}\%$ of bichromate of potash, the percentage of solids was found as follows :—

TABLE 7.

SERIAL NUMBER.	PERCENTAGE OF SOLIDS.
70	19.3
71	19.5
72	19.1
73	19.5
Average	19.3

When we consider Table 7, we see that a $\frac{1}{2}\%$ solution of bichromate of potash reduces the quantity of solids slightly below the normal.

The reduction in this instance must be the result of the extraction of the solids from the specimen by the solution. In Table 6, the increase in solids certainly comes from the salts, and we see that in these cases the increase in solids is less than is to be expected, if the fluid within the specimens contains the same percentage of salts as that surrounding it. That this percentage of salts increases with time, is also shown in this Table 6, but as yet we have no evidence to show to what extent the 2% solution of bichromate of potash extracts solids from the specimen, and we therefore cannot say to what degree the result obtained is a balance struck between opposing processes.

As the following Table 8 shows, the increase in weight is dependent to some degree on the compressing action of the pia on the specimen ; and in this connection the reaction of specimens with the pia intact and those with the pia slit, have been studied. Where the pia is slit the percentage of solids is greater.

Specimens of entire encephala hardened for 41 days in a 2% solution of bichromate of potash:—

TABLE 8.

SERIAL NUMBER.	PERCENTAGE OF SOLIDS.	AVERAGE.	PERCENTAGE INCREASE IN WT. AFTER 41 DAYS.
108 (Slit)	21.4	21.0	39.5
109 “	20.6		40.7
110 (Intact)	20.3	20.1	35.7
111 “	19.9		36.2

The following Table 9 shows the same relation as regards the percentage of solids. In addition it shows that in specimens whether slit or not, a temperature of 37° C. increases the percentage of solids.

Specimens of entire encephala hardened for 35 days in a 2% solution of bichromate of potash:—

TABLE 9.

SERIAL NUMBER.	PERCENTAGE OF SOLIDS.	AVERAGE.
105 (Slit)	23.3 ¹	} 21.5
106 “	21.4	
107 “	21.7	
102 (Intact)	23.1 ¹	} 21.0
103 “	20.7	
104 “	21.3	

¹ These specimens were put for 12 days at a temperature of 37° C. Not only do the slit specimens show a larger percentage of solids, but placing the specimens both slit and entire, at 37° C., increases the solids and gives the greater increase again in the specimens slit. The action of heat was followed in the way shown below.

Table 10 left hemiencephala after hardening in a 2% solution of bichromate of potash for 35 days :—

TABLE 10.

SERIAL NUMBER.	PERCENTAGE OF SOLIDS.	AVERAGE.
96	22.0	= 21.4
97	20.8	

Table 11 left hemiencephala hardened 35 days in a 2% solution of bichromate of potash at ordinary temperature, then heated in an oven at 37° C. for 30 days :—

TABLE 11.

SERIAL NUMBER.	PERCENTAGE OF SOLIDS.	AVERAGE.
51	23.9	= 23.2
52	22.6	
53	22.6	
54	24.5	
55	22.7	

This shows very nicely the effect of the higher temperature on the percentage of solids.

The percentage of solids found in Table 11 is above that which would come from mere saturation of the fluid within the specimen ; hence, at 37° C., under the above conditions, we must have some deposit of salts in addition.

As a result of these observations, we have determined that in the bichromate of potash solutions the specimen takes up both water and salts as it becomes hardened in the histological sense.

Variation.—In this connection, it needs but a glance at some of the foregoing tables to show that although the attempt has been made to have the conditions similar in different series, the results may vary by as much as 8%.

In search of an explanation for this, we have made the observations which immediately follow.

Age.—In our experiments the age of the animals, which were obtained from the slaughter-house, was inferred from the weight of the brain. They were mainly wethers from the Cotswold breed of sheep.

A series of 52 sheep's brains gives the following statistics : The heaviest hemiencephalon was 69.65 grms., and the lightest, 43.17 grms. The left hemiencephalon in the case of 40 brains averaged 56.4 grms., and the right hemiencephalon 56.1 grms. The so-called lambs' brains were 12% lighter.

The attempt was repeatedly made to determine whether the fresh weight of the sheep's brain or the size of the bosses on the skull, both considered as indices of age, bore any relation to the amount of the increase in weight in the bichromate solutions, but no such relation could be found.

Neither was it found that the percentage of solids was correlated with the fresh weight of the brain.

We conclude, therefore, that the differences in brain weight due to the ages within which our specimens ranged, are not significant for our purpose, nor is the difference above noted in the weights of the two halves of the encephalon indicative of anything, save the difficulty of dividing the brain evenly.

Season.—The brains were placed in the hardening solutions at different times of the year without finding any variations to be associated with the seasons.

Influence of Pressure.—Of the minor conditions which influence the change in weight, pressure, as we have just pointed out, in speaking of specimens with the pia intact and those with it slit, is the most important. If a specimen be pressed against the containing vessel, or bound about in any way, two things happen,—it swells less than under ordinary conditions and hardens more slowly or not at all, according to the degree to which it is compressed.

The specimens were all treated without removing the pia. The pia mater is but a slightly extensible membrane, consequently when an encephalon with the pia intact is put into a solution of bichromate of potash, it does not increase in weight so much or harden so quickly as another encephalon in which the pia has been slit.

In this latter case, the brain substance bulges out at the slits in a way which suggests that it has been restrained in swelling by the action of the surrounding membrane.

Influence of Slits.—The matter was repeatedly tested in this way. A series of encephala were carefully removed with the pia intact; then half the number of specimens were slit with a scalpel, so as to largely do away with the restraining action of the pia. In the first series six encephala were taken,—three intact, three slit.

At the end of 48 days, in a 2% solution of bichromate of potash, we have the following :—

TABLE 12.

SERIES I.

Percentage increase in weight at the end of 48 days :—

BRAIN WITHOUT SLITS.	BRAIN WITH SLITS.
28.9	36.8
26.4	33.8
32.0	38.6
Average = 29.1	Average = 36.4
	Excess = 7.3

SERIES II.

At the end of 24 days :—

27.4	37.4
32.0	39.6
26.9	36.1
Average = 28.8	Average = 37.7
	Excess = 8.9

SERIES III.

	AFTER 8 DAYS.	AFTER 24 DAYS.	AFTER 41 DAYS.
Encephala without slits .. {	28.2	34.6	35.7
	27.1	34.7	36.2
Average	= 27.6	= 34.6	= 36.0

SERIES III—CONTINUED.

	AFTER 8 DAYS.	AFTER 24 DAYS.	AFTER 41 DAYS.
Hemiencephalon, right . .	31.8	36.6	38.7
“ left . .	32.5	38.3	38.4
Average	= 32.1	= 37.5	= 38.5
Encephala with slits . . }	38.1	41.2	39.5
	38.5	40.8	40.7
Average	= 38.3	= 41.0	= 40.1

In the second series we have a similar relation developed at the end of 24 days.

On considering the third series presented, it will be seen that in all cases the slit brains attained a greater weight than those intact, and that the hemiencephala occupy in this respect an intermediate position. They must of course be considered as specimens in which the restraining action of the pia is in part removed by the hemisection.

When the three portions of the third series are compared, it is further seen that the intact encephala increase more slowly and for a longer time than those which are slit, but that at any given date the slit specimen is always the heavier.

There is no doubt that this pressure of the pia on the swelling nervous substance plays an important rôle in bringing about the differences between the specimens, such as we find in our earlier series; but at the same time it is not an explanation for all the cases, for in many specimens where the weights after hardening were dissimilar enough to excite attention, and which were later examined in the light of these results, there could not be found a sufficient difference in the slitting of the pia to make the above explanation adequate.

In view of these facts, however, we can understand why a small piece of nervous tissue should harden better than a larger one, and why the central portion of a large piece, by reason of the pressure from the external layers already swollen by the hardening fluid on its way to the interior, as well as

from the late arrival of the denser fluid, should often remain quite unhardened.

Freshness.—It appears also that the time elapsing after death has a decided influence on the increase in weight.

The series which we quote was tested with a 2% solution of bichromate of potash to which had been added one sixth of its volume of 94% alcohol. The reaction of this solution is similar to that of the simple bichromate of potash, but it causes a decidedly smaller increase in weight.

This difference in treatment does not prevent the results from being applicable in this connection. Two control hemiencephala were used. Three others were removed and allowed to remain in covered dishes at the room-temperature for the times indicated in the following table :—

TABLE 13.

STATE.	INITIAL WEIGHTS.	PERCENTAGE CHANGE IN WEIGHT 16 DAYS.	MEAN TEMPERATURE. JULY, 1890.
Fresh.	57.6 grms.	+ 20%	
"	63.8 "	+ 21	14th, 20° C.
Stale 1.3 days.	56.5 "	+ 0	15th, 24° C.
" 1.8 "	54.7 "	— 2	
" 2.05 "	56.5 "	— 1.5	16th, 26° C.

The specimens did not harden after they had been allowed to become stale. Neither did they swell. These figures leave no doubt that shortly after death changes may take place in the nervous structures which prevent it from increasing in weight in the above solution.

We have several other series which suggest the same thing.

Table 4, given to illustrate the effects of using different quantities of the hardening solution, can be placed in evidence of this point, since nearly 7 hours elapsed between putting the first and the last pairs of specimens into the fluid, and the intervening pairs were put in at intervals of 2 hours, in the order in which they stand.

It will require a special investigation to determine just the course of this loss in the power to increase in weight, and also

to determine whether the process goes on in the same manner in a brain remaining within the skull as in a brain which has been removed.

Parts of Encephalon.—Up to this point we have been considering the reactions of the subdivisions of the brain taken in the normal proportions, as represented in a hemiencephalon.

We shall now take up separately the several parts of the brain. The subdivisions examined were as follows:—

The stem, consisting of the oblongata, pons and quadrigemina. The cerebellum, separated from the stem at the base of its peduncles; and the cerebrum, the portion lying in front of the quadrigemina. The cerebral hemispheres were without the gray caps of the olfactory lobes and without the hypophysis.

After 153 days in 2% bichromate of potash the weight relations were as follows:—

TABLE 14.

	R. HEMISPHERE.		L. HEMISPHERE.	CEREBELLUM.		STEM.		
SERIAL No.	FRESH WEIGHT.	PER- CENTAGE GAIN.	PER- CENTAGE GAIN.	PER- CENTAGE GAIN.		PER- CENTAGE GAIN.		
82	42.80	+ 35.1	44.50	+ 37.5	12.25	+ 29.3	19.61	+ 32.8
83	41.55	33.1	39.73	31.6	13.00	28.1	20.03	29.7
84	32.55	32.7	32.69	33.4	12.07	26.8	18.43	32.9
	Avg. = 33.6		= 34.1		= 28.1		= 31.8	

Average of both Hemispheres = 33.8.

From the consideration of these figures we see that the cerebrum increases most in weight, next the stem, and last the cerebellum. These parts of the brain represent varying mixtures of gray and white matter, and for this reason might be expected to react differently. From tests made for the purpose it has been found, as might be expected, that the increase in the weight of the gray matter is only about half of the white. Exact figures have little value in this instance, because gray matter can never be obtained pure, and its increase in weight is modified by the proportion of white substance in it. In this

instance the gray matter was the cortical gray of the sheep, which, of course, contains a large proportion of white matter. A determination of the percentage of solid matters in the subdivisions of the fresh encephalon yielded the following:—

TABLE 15.

Percentage of solids in the subdivisions of the fresh encephalon:—

SERIAL No.	R. HEMISPHERE.	L. HEMISPHERE.	CEREBELLUM.	STEM.
98	21.9	20.3	21.5	25.6
99	21.2	20.0	18.9 ¹	25.6
74	21.0	20.6	20.9	27.3
75	21.0	21.4	21.0	26.7
101	20.9	20.2	18.9 ¹	26.3
87	20.8	21.0	21.5	27.3
86	20.3	20.4	20.2	26.7
	Avg. = 21.0	= 20.5	= 21.0	= 26.5

As will be seen, this shows the percentage of solids in both hemispheres and in the cerebellum to be about the same, while the percentage in the stem is much higher.

Taking the percentage of solids as an index of the proportion of white matter, and remembering that under the influence of bichromate of potash solution, the white matter increases most in weight; we should expect that the order in which the subdivisions would increase in weight, would be the stem most, and the cerebrum and cerebellum in about the same degree.

As a matter of fact under the conditions of our experiments, the cerebellum increases least, the stem next, and the cerebrum most. The strong system of supporting tissues in the stem is doubtless one influence acting against its greater enlargement. Whether the laminated condition of the white matter in the cerebellum, is a cause of the failure to increase in weight, or whether the composition of the medullary substance is there different, has yet to be determined. As we shall see when

¹ The low percentage of the solids in the cerebellum in the instances indicated is sufficiently noticeable to call for explanation, but at present none can be given. The average for the solids of the cerebellum without these two low figures is 21.0%. With them it is 20.4%.

discussing the reactions of the human brain, too much weight should not be laid on the differences just noted.

Though solutions of bichromate of potash are most frequently used in hardening the brain, yet alcohol also plays an important rôle, either alone or in connection with the bichromate of potash solution. Moreover a study of the action of alcohol may help us to understand the manner in which the change in weight occurs and the conditions influencing it.

It will be best, therefore, to take up at this point the action of ethyl alcohol on the encephalon of a sheep. As before we take a typical specimen.

TABLE 16.

Hemiencephalon of a sheep in 500 c.c. of 95% Ethyl Alcohol.

TIME IN DAYS.	PERCENTAGE LOSS.
1.2	19.1
2.2	24.3 (c)
3.8	31.8
5.9	32.9
6.9	33.9
32.0	33.5
62.0	34.2
105.0	34.4
198.0	36.2
550.0	36.5
636.0	36.3

The great rapidity with which weight is at first lost, and the long period through which a slow loss continues, are both features of interest. The total loss in weight in this percentage of alcohol is almost equal to the total gain in the $2\frac{1}{2}\%$ solution of bichromate of potash.

The whole method of preparation and weighing, as described for the specimens in the bichromate, was followed in the case of the observations with alcohol. Thus far we have used ethyl alcohol only. The percentage of the alcohol was always determined by the Tralles scale.

Percentage of Alcohol.—Under the action of alcohol the decrease in weight is less when the alcohols of the lower percentages are used. This is shown in the following table:—

TABLE 17.

Change of weight after 70 days.

ALCOHOL.	PERCENTAGE DECREASE.
95%	34.2
"	33.4
93%	32.0
"	33.2
80%	20.3
"	21.6
70%	13.8
"	14.8
60%	9.8
"	9.5
50%	0.04
"	0.05

It is interesting to note, that in the case of the 50% alcohol the loss in weight recorded, is preceded by an increase in weight, *i. e.*, a period during which the specimen is swollen. This increase lasts only a day or two and then gives place to a decrease.

The cause of the decrease in weight is due to a decrease in the volume of the specimen accompanied by an extraction of the solids. For example, after 245 days, in 95.5% alcohol, specimen C., for which the figures are given in Table 16, had an absolute weight of 33.3 grms. and a volume of 37.8 c.c.; at this time it showed a loss of some 32% in weight. The volume of the specimens in alcohol is greater than the weight, while for the specimen in bichromate of potash, as for the fresh brain, the volume is less than the weight. To this condition, of course, the low specific gravity of the alcohol contributes. Considered as a sponge, the specimen holds absolutely less fluid in the alcohol, than when in its normal condition. The contained fluid is lighter, and further the alcohol according to its strength has extracted a larger or smaller proportion of solids.

Volume.—Before discussing the extraction of solids by alcohol, we may briefly state some observations made on the volume of the brain in various stages of hardening, as we have already had occasion to allude to the matter. At the beginning

of this series of observations it was planned to make throughout a series of determinations of the volume parallel to those for weight. For this purpose a special apparatus was devised and found to work with accuracy. As we do not propose to go into the detail of this subject, the apparatus need not be described at this time.

The outcome of the observations was that the curve for volume ran parallel, or nearly so, with that for weight.

In this connection but one detailed observation was made and this with the purpose of determining whether in specimens hardened in alcohol, changes of temperature between 10° C. and 30° C. continued for an hour, caused variations in the volume. No change in volume was found, whereas specimens hardened in bichromate of potash, at the normal temperature or below, and then placed at 37° C., for some days undergo a decided decrease in volume, accompanying the decrease in weight.

Period of Maximum.—Just here something may be said concerning the attainment of the maximum increase or decrease in weight.

The terminal weight after long hardening by no means always represents the maximum departure from the normal. It appears that within the limits of our observations the higher temperature favors the early attainment of a maximum. That in those specimens that attain the maximum at an early date the return toward the normal is most marked. When we come to study the human brain we shall find that renewal of the solution of the bichromate of potash is often followed by a decided decrease in the weight and volume of the specimen. The materials on which the change in weight depends, are therefore capable of variations in reaction even after long immersion in the hardening fluid.

Extraction of Solids by Alcohol.—From this digression we return to the consideration of the action of alcohol on the solids of the specimen. We take the normal percentage of solids in the fresh encephalon to be 20.5%. We find that the alcohol employed for hardening contained 0.004 grms. of solids in each 100 c.c. of fluid. For our purposes this is insignificant.

TABLE 18.

Determination of solids extracted.

Six encephala after 55 days in 95% alcohol showed a percentage of solids based on the fresh weight, as follows :—

SERIAL NUMBER.	PERCENTAGE OF SOLIDS.
76	15.4
77	15.8
78	15.1
79	15.6
80	15.1
81	13.8
<hr/>	
Average	15.1

Six hemiencephala after 60 days in 95.7% alcohol.

SERIAL NUMBER.	PERCENTAGE OF SOLIDS.
64	13.4
65	11.2
66	12.9
67	13.9
68	13.6
69	14.9
<hr/>	
Average	13.3

We have no observations on the power of the several percentages of alcohol to extract solids, nor on the time relations of this power, but it would appear from the above figures that the extraction is still going on between the 55th and 60th days, when the decrease in volume has reached nearly a maximum.

Recovery of Solids. — The loss of the solids due to the action of alcohol was controlled, by weighing the solids after evaporating known quantities of the alcohol in which the specimens had been hardened. The control was satisfactory. These facts show that nervous tissues are damaged by the action of strong alcohol through the extraction of solids.

To return now to the conditions which control the change in weight. Since specimens from different animals treated in the same manner did not change weight to the same degree, it might be supposed that either the specimens differed as

regards the amount of restraint exerted on them by the supporting tissues, or that they differed as regards the proportion of substances particularly responsible by their swelling for the increase in weight, or in both these ways. In case it were the former, since the supporting structures are at the same time inelastic and shrinkable by alcohol, it might be assumed that the specimens which increase least in weight, under the action of the 2% solution of bichromate of potash, would be those which would decrease most under the action of strong alcohol, for the supporting tissues would resist the swelling by the bichromate and effect the contraction under alcohol. The experiment was made, six encephala being taken. The six right halves were put into 2400 c.c. of 2% bichromate of potash solution, the six left halves were put into 2400 c.c. of 94.5% alcohol. At the end of 65 days those in bichromate of potash had gained, and those in alcohol had lost as follows :—

TABLE 19.

SERIAL NUMBER.	PERCENTAGE GAIN IN BI. POT. 2 PER CENT.		PERCENTAGE LOSS IN ALCOHOL 94.5 PER CENT.	PERCENTAGE OF SOLIDS FOUND IN ALCOHOL SPECIMENS.
	CORRESPONDING HALVES.			
1	1'	35.8	30.2	13.6
2	2'	34.4	29.6	13.9
3	3'	33.5	29.5	13.4
4	4'	32.8	29.9	11.2
5	5'	32.7	28.8	12.9
6	6'	32.4	27.7	14.9

It is evident from this table that while the correspondence is not complete in detail, yet there is a correspondence of such a nature that those specimens which increase most in bichromate of potash also decrease most in alcohol.

This suggests that there is a simple explanation for the variations exhibited by the reactions of different specimens, but it also indicates that the hypothesis that the difference is due to the reaction of the supporting tissues alone, does not account for the facts. The explanation is by no means evident, since in the case of the alcohol specimens, those which have

lost most in weight are not those which have lost the greatest percentage of solids, so that the final weight of the alcohol specimens is the resultant of both shrinkage and extraction of solids, and it is not clear how these processes are related to each other.

Effects of Bichromate of Potash.—From all the foregoing observations we may conclude :—that the sheep's brain, under the influence of solutions of bichromate of potash, increases in weight and volume ; that by far the greatest change is during the first 24 hours ; that after the first two weeks the increase is slight and comparatively insignificant ; that after the maximum weight is attained there may occur a slight decrease. The gain in weight is made greater by freshness, absence of pressure, and low percentage of salts in the solution, it is made less by a temperature of 38° C. The increase in weight is due to the taking up of the solution by the specimen.

The fluid within the specimen at first contains a smaller percentage of the salt than is present in the surrounding fluid.

The percentage of solids may be increased above the normal, by prolonged hardening in a solution of 2% or more of bichromate of potash. A temperature of 38° C. increases the percentage of solids still further.

Effects of Alcohol.—The general action of alcohol is to decrease the weight and volume of the sheep's brain. The rate at which this takes place is similar to that for the increase in bichromate of potash.

The higher the percentage of alcohol, the more rapid and greater is the loss in weight. The loss in weight is due to the decrease in the volume of the specimen by shrinkage, extraction of solids and replacement of water by the alcohol of a less specific gravity. In the case of 50% and 60% alcohol, the final decrease in weight is slight, and is preceded by an increase.

Strong alcohol decreases the percentage of solids. — So long as the exact time of death is not controlled, we shall be unable to determine how far the changes in weight in a given specimen are due to changes dependent on post mortem alterations, and how far they represent ante-mortem conditions.

Additional Observations. — Before leaving the sheep, we have yet to mention some of the most common methods of hardening in which modifications of the simple bichromate solutions are employed, or where two different fluids are used in succession.

On making an average of the changes in four series of hemiencephala in a $2\frac{1}{2}\%$ solution of bichromate of potash we have the following figures shown in the next table.

This may be taken as a standard.

TABLE 20.

Average increase of 4 hemiencephala in $2\frac{1}{2}\%$ solution of bichromate of potash.

TIME.—DAYS.	PERCENTAGE INCREASE.
1.05	18.8
2.20	24.8
3.12	31.3
5.12	33.0
12.12	36.0

The maximum increase in any of these four specimens during the next 660 days is less than 2%, so that the table presents fairly well the resulting increase in weight.

If we compare with this a solution containing the same percentage of bichromate of potash to which $1\frac{1}{2}\%$ of sodium sulphate has been added, the increase in weight is found to be smaller. *Vide* the following table :—

TABLE 21.

Average increase of 4 hemiencephala in $2\frac{1}{2}\%$ solution of bichromate of potash plus $\frac{1}{2}\%$ sulphate of soda.

TIME.—DAYS.	PERCENTAGE INCREASE.
.9	14.2
1.0	16.5
2.9	24.5
3.0	28.6
5.1	30.5
7.0	31.9
12.0	32.0
41.0	33.2

In the same way the addition of $\frac{1}{6}$ its volume of alcohol to the bichromate of potash solution, reduces the percentage of increase, as follows :—

TABLE 22.

Influence of $2\frac{1}{2}\%$ solution of bichromate of potash to which $\frac{1}{6}$ its volume of 95% alcohol has been added.

TIME.—DAYS.	PERCENTAGE INCREASE.
1.1	16.6
2.2	21.5
3.9	23.6
5.9	25.5
12.9	26.4
32.0	28.2

In this connection it is of interest to note that the percentage of the bichromate of potash solution is effective in restraining the increase in weight even in combination with alcohol, Tables 23, 24. In these latter tables we are dealing with entire encephala in which the pia is intact, and so it happens that in Table 23, the increase in weight under the $2\frac{1}{2}\%$ bichromate of potash, is less than in the Table 22 just given, where hemiencephala are employed.

TABLE 23.

Observations on two encephala in a solution of $2\frac{1}{2}\%$ bichromate of potash plus $\frac{1}{6}$ its volume of 95% alcohol.

PERCENTAGE INCREASE AFTER 75 DAYS.	
19.2	20.9

TABLE 24.

Observations on four encephala after 75 days in a solution of 2% bichromate of potash plus $\frac{1}{6}$ its volume of 95% alcohol.

PERCENTAGE INCREASE.			
24.7	24.7	24.9	26.1

When $\frac{1}{2}\%$ of copper sulphate is added to the solution and the specimens are kept at 38° C., we get the combined action of a high temperature and the copper sulphate as exhibited in Table 25.

The period of loss of weight in these cases follows rapidly on the attainment of the maximum.

TABLE 25.

Average of two hemiencephala in a solution of $2\frac{1}{2}\%$ bichromate of potash plus $\frac{1}{2}\%$ sulphate of copper, kept at 38° C.

TIME. — DAYS.	PERCENTAGE INCREASE.
1.12	13.0
3.9	14.2
5.9	15.1
10.9	14.4

Nitric acid and zinc chloride were also tried.

TABLE 26.

Average of observations on two hemiencephala in 980 c.c. aq. dist. plus 20 c.c. nitric acid, sp. gr. 1.42.

TIME. — DAYS.	PERCENTAGE CHANGE.
.9	+ 7.1 (c.)
3.09	+ 1.0 (c.)
5.8	— 1.0 (c.)
12.09	— 2.4

TABLE 27.

Average of observations on two hemiencephala in 1000 c.c. of a saturated solution of zinc chloride, in 95% alcohol.

TIME. — DAYS.	PERCENTAGE DECREASE.
0.9	13.8
3.2	21.4
5.9	24.1
9.9	25.5 (c.)

Nitric acid in the proportion given, certainly causes very little disturbance in the weight of the sheep's brain, while zinc chloride dissolved in strong alcohol, produces a decided loss of weight — almost as great as that found from alcohol alone.

Below in Table 28 is given a series of fluids in which the entire encephala of the sheep were placed for 48 hours. During this interval the specimens were weighed five times. The terminal weights are alone given in order to show the general action of the fluids employed.

TABLE 28.

SOLUTION.	PERCENTAGE CHANGE AFTER 2 DAYS.	CONDITION OF SPECIMEN.
Mercuric Chloride 1:10,000.	+ 48.7%	Very soft.
Tap Water.	+ 38. %	Soft.
Alcohol 50%.	+ 3.8%	—
Air at 20° C.	— 0.9%	—
Sodium Chloride. Sat. Solution in Water.	— 5.0%	Soft.
$\frac{1}{2}$ vol. 95% Alcohol, $\frac{1}{2}$ vol. Glycerine.	— 25.0%	Hard.

TABLE 29.

A series of hemispheres were subjected to the conditions below indicated :

SOLUTION.	PERCENTAGE CHANGE.			CONDITION OF SPECIMEN.
	AFTER 3 DAYS.	AFTER 9 DAYS.	AFTER 180 DAYS.	
5% Sugar Solution in Water.	+ 1.8%			Soft and Pink. Mouldy.
25% " " " "	+ 2.0%	— 14		
5% Tartaric Acid " " (2 days).	+ 14.0%			Very Soft. Soft and Pink.
{ 1 vol. 4% Bichromate of Potash. } R. II.	+ 2.1%		— 2	
{ 1 " 95% Alcohol. } L. II.	+ 1.9%		— 4	
5% Potassium Tartarate.	— 1.4%	+ .7		
5% Sodium Sulphate.	— 7.8%			
5% Zinc Chloride Solution in Water.	— 9.0%		— 16	
25% " " " " "	— 17.0%		— 30	

The specimens in Table 29 were hemispheres only, and hence differed slightly from those in Table 28. The difference does not in all probability affect the general results.

Special attention is called in Table 29 to the action of the solution containing equal volumes of 4% bichromate of potash and of 95% alcohol, since the final change in the specimen is slight. Judging from the figures above given, 50% alcohol, a saturated solution of sodium chloride, as well as the bichromate of potash and alcohol mixture just mentioned, disturb the normal weight but slightly. We come, finally, to the reaction which follows the treatment with two successive fluids. After ample hardening in bichromate of potash, alcohol produces more or less loss of weight.

TABLE 30.—OBSERVATIONS ON HEMENCEPHALA.

FIRST SOLUTION.		TIME. DAYS.	PERCENTAGE CHANGE AFTER 1ST SOLUTION.	SECOND SOLUTION	TIME. DAYS.	PERCENTAGE CHANGE AFTER 2ND SOLUTION.	PERCENTAGE OF SOLIDS.
2½% bichromate of potash	60	+ 34.9	80% alcohol	840	+ 4.9	20.5
" " " "	105	+ 34.7	70% " "	900	+ 6.0	19.8
" " " " plus ½ its volume of 95% alcohol	60	+ 28.7	80% " "	840	+ 1.8	19.7
2½% bichromate of potash plus ⅔% copper sulphate at 38° C.	13	+ 17.0	80% " "	960	0	20.2
" " " " " sodium sulphate.	60	+ 29.0	80% " "	840	— 2.5	18.4
" " " " " "	105	+ 33.7	70% " "	900	+ 6	19.3
2% bichromate of potash	56	+ 32.2	500 c.c. 95% alcohol	41	— 7	16.7
" " " " at 38° C.	56	+ 23.3	500 " " "	41	+ 5.8	18.5
" " " " " "	56	+ 36.0	500 " " "	41	— 3	16.7
" " " " " "	60	+ 32.8	500 " " "	41	+ 1.4	17.7
" " " " " "	60	+ 37.0	500 " " "	41	+ 2.2	17.7
" " " " " "	60	+ 37.0	500 " " "	41	+ 1.6	17
4% " " " " " "	88	+ 23.7	70% alcohol	810	+ 2	
8% " " " " " "	88	+ 14.1	70% " "	900	— 9	
2½% bichromate of potash plus ⅓% copper sulphate at 38° C.	13	+ 13.6	1% bi. pot. & camphor	990	+ 16	
980 c.c. water plus 20 c.c. nitric acid sp. gr. 1.42	15	+ 3.4	1% " "	990	— 2	
Saturated solution zinc chloride in 95% alcohol	10	— 25.5	Glycerine	990	— 0.5	
93% alcohol	12	— 34.4	After draining	45	— 24	15
" " " " " "	12	— 32	70% alcohol made by add- ing water to alcohol in use	990	— 26	16.5
60% " " " " " "	88	— 10.7	94% alcohol	930	— 29	

It will be at once seen, that alcohol reduces the weight of the hardened specimen in the same way that it does that of a fresh one, and that while alcohol under 80% can produce a considerable loss of weight, due principally to shrinkage, the higher grades may also extract nearly $\frac{1}{5}$ of the total solids.

SECTION II. — *Shark's Brain.*

During the summer of 1890, the facilities offered by the Marine Biological Station at Wood's Holl, enabled us to test some of these reactions on the brains of several specimens of shark.

The subjoined Tables 31-33 give the principal results, and indicate that the reaction of the shark's brain is similar to that of the sheep.

TABLE 31.

Average of two specimens, *Galeus canis*, — average fresh weight — 7.05 grm.
Fresh in 2½% solution of bichromate of potash.

Ditto 6.05 grm. allowed to stand in a covered dish for 36 hours, then put into 2½% solution of bichromate of potash. Mean daily temperature, 21.6° C.

TIME. — DAYS.	PERCENTAGE INCREASE. FRESH.	PERCENTAGE DECREASE STALE.
0.9	39.4	— 2%
2.3	47.4	
7.2	49.2	2.5
18.9	49.0	3.2
338.	47.1	5.5
432.1	46.3	3.5

TABLE 32.

One specimen (*Sphyrna zygaena*), fresh weight 25.9 grms. Fresh in $2\frac{1}{2}\%$ solution of bichromate of potash plus $\frac{1}{5}$ its volume of 95% alcohol.

Ditto, fresh weight 28.05 grms., allowed to stand about 24 hours. Put in the same solution. Mean daily temperature, 18.9° C.

TIME. — DAYS.	PERCENTAGE INCREASE. FRESH.	PERCENTAGE INCREASE. STALE.
1	+21.4	+ 1.3
1.25	27.2	3.5
2.25	28.7	8.1
95.	33.9	8.3
323.	38.5	15.9
426.	40.2	17.2

TABLE 33.

Average of two specimens (*Galeus Canis*). Average fresh weight 5.6 grms. Fresh, in 90% alcohol.

TIME. — DAYS.	PERCENTAGE LOSS.
0.9	— 25.5
2.18	31.9
7.1	33.3
18.45	33.6
111.	33.1
338.	37.0
432.	31.3

The tables show that the bichromate of potash solution and the alcohol produce a similar reaction to those obtained from the sheep's brain.

The influence of post-mortem degeneration is also clear.

SECTION III. — *Human Brain.*

The interest of the results obtained on the brain of the sheep will largely depend on their applicability to human material. In the comparison the following data are employed. For the percentage of water we take Thudichum's figures :—

Mean for the cortical gray substance =	85.27%
Mean for the white substance, cerebrum =	70.23%

For the solids the corresponding figures :—

Mean for cortical gray substance =	14.73%
Mean for white substance, cerebrum =	29.77%

As is plain, the solids in the white substance are twice as abundant as in the gray. We begin with the observations on the adult—all the brains being from persons more than 25 years of age. Unless otherwise stated, 4 litres of fluid were always used. The letter (c.) after an entry in the table means as previously stated, that the fluid was changed, *i.e.* renewed at the time of the observation after which it stands. As in the case of the sheep's brain, the pia was not removed.

If a human encephalon without cutting in any way, be put in 2% bichromate of potash, the following is the result :—

TABLE 34. — BRAIN XVIII.

Female. Age, 40 years. Death from pneumonia. In the hardening fluid 16 hours after death. 2% solution of bichromate of potash. Encephalon intact. Fresh weight 1335 grms.

TIME. — DAYS.	PERCENTAGE INCREASE.
0.7	6.2
120.0	25.2
270.0	24.2

It can be seen by consulting Tables 35 and 36 that subdivision of the encephalon, before putting it into the hardening fluid, is followed by a greater change in weight.

TABLE 35. — BRAIN XIX.

Male. Age, 22 years. Death from phthisis. In the hardening fluid 11 hours after death. 2% solution of bichromate of potash. Encephalon subdivided. Fresh weight 1248 grms.

TIME. — DAYS.	PERCENTAGE INCREASE.
120	34

The figures show clearly enough the influence of subdividing the encephalon on the subsequent reaction. In the larger number of the tables which follow, the changes in the subdivisions of the encephalon are recorded separately.

We shall now present a series of tables giving the change of weight under the action of various fluids.

The tables have several uses. They show in the main the similarity of the reactions in the case of man and the sheep, a similarity which is close enough to justify us in using the sheep's brain for further investigation.

They offer records from which the fresh weight of hardened human brains can be in some cases inferred, and they emphasize certain peculiarities in the reactions which appear to belong to the human encephalon by virtue of its larger size.

TABLE 36. — BRAIN X.

Male. Age, 35 years. Death from diffuse nephritis. In hardening fluid 24 hours after death. $2\frac{1}{2}\%$ solution of bichromate of potash. Encephalon subdivided into two cerebral and two cerebellar hemispheres and the stem. Subdivision as in the sheep. Each portion weighed separately. In the tables, the average changes in the cerebral and cerebellar hemispheres are alone given.

TIME.—DAYS.	PERCENTAGE INCREASE.		
	HEMISPHERES.	CEREBELLUM.	STEM.
2.1	20.1	19.3	17.2
7.2	26.0	29.1	22.0
30.	32.3 (c.)		28.5 (c.)
74.	32.3	28.7	28.5
96.	(c.)		
322.	31.2		
539.	31.4	32.2	30.2

This shows a less increase than was found in Table 35, where the encephalon was also subdivided, but here a solution of $2\frac{1}{2}\%$ bichromate of potash was employed. In man, therefore, as in the case of the sheep, the higher percentage of salts causes the smaller increase in weight.

TABLE 37. — BRAIN XI.

Female. Age, 40 years. Death from phthisis. In hardening fluid 15 hours after death. $2\frac{1}{2}\%$ solution of bichromate of potash. Encephalon subdivided as in Table 36, except that the cerebellum was entire.

TIME.—DAYS.	PERCENTAGE INCREASE.		
	HEMISPHERES.	CEREBELLUM.	STEM.
.9	9.0	05.1 (c.)	07.7
5.7	21.0	14.9	21.1
29.9	27.7 (c.)		25.5 (c.)
72.	27.6	18.5	24.6
96.	(c.)	(c.)	(c.)
330.	28.6	19.5	23.9
532.	30.1	21.8	28.3

Probably the fact that the cerebellum was not subdivided, is one reason why it did not increase more in weight.

TABLE 38. — BRAIN III.

Male. Age, 39 years. Death from phthisis. In hardening fluid 10 hours after death. $2\frac{1}{2}\%$ solution of bichromate of potash plus 1% of sodium sulphate. Encephalon completely subdivided.

TIME.—DAYS.	PERCENTAGE INCREASE.		
	HEMISPHERES.	CEREBELLUM.	STEM.
0.9	3.0	4.1	2.5
2.7	3.3	7.2	1.2
5.7	7.1	12.0	6.9
9.8	8.6	10.9	7.0
13.8	9.1		8.8
18.	(c.)	(c.)	(c.)
32.	8.5		6.3
75.	7.2	9.8	5.9
110.	(c.)	(c.)	(c.)
342.	8.3		9.5
512.	9.1	13.1	10.8

By an oversight, the sodium sulphate in the solution used on the sheep's brain was only $\frac{1}{2}\%$ instead of 1%, so that these

figures are not directly comparable with the observations on the sheep.

TABLE 39.—BRAIN XII.

Male. Age, 35 years. In hardening fluid 30 hours after death. 2½% solution of bichromate of potash, plus 1% of sodium sulphate. Encephalon subdivided, except the cerebellum.

TIME.—DAYS.	PERCENTAGE INCREASE.		
	HEMISPHERES.	CEREBELLUM.	STEM.
1.0	6.0	6.0	5.0
4.9	10.0		11.1
7.0	(c.)	(c.)	(c.)
30.05	10.9	12.0	10.4
72.	8.4	12.2	7.7
92.	(c.)	(c.)	(c.)
329.	8.3	11.7	9.1
531.	9.6	13.	9.4

In the last two tables we have shown the restraining effect of the 1% of sodium sulphate. The cerebellum appears least affected by this new condition.

TABLE 40.—BRAIN XIII.

Male (negro). Age, 60 years. In hardening fluid 20 hours after death. In 8% solution of bichromate of potash. Encephalon completely subdivided. One cerebral and one cerebellar hemisphere and the stem were used for this test.

TIME — DAYS.	PERCENTAGE INCREASE.		
	HEMISPHERES.	CEREBELLUM.	STEM.
.8	4.9	4.8	5.3
3.8	10.1	10.4	10.7
5.	(c.)		(c.)
30.	16.7		16.2
70.	15.3	15.0	15.6
328.	17.5	16.7	17.5
530.	19.4	20.8	21.1

The comparison of the figures, Table 40, with those for the sheep, is to be made with due allowance for the fact that an average human encephalon contains from 900 to 1000 c.c. of fluid, and that when it is placed in 4000 c.c. of an 8% solution of bichromate of potash, the ultimate effect is to reduce the percentage of this solution $1\frac{1}{2}\%$. This we know is a significant reduction, and greater than took place in the case of the sheep's brain, since there the reduction in a solution ten times the weight of the specimen, is only about 0.6%.

As matter of fact, the jars generally available in our laboratories do not admit of adding more than five or six litres of fluid to an entire brain, and even less than this amount is ordinarily used; so that in the case of the human brain especially, the amount of fluid employed is of much significance, in determining the change in the weight of the specimen. This fact may account for the advice repeatedly given in the books to frequently change fluids, since this, for one thing, at least, keeps up the percentage of salts.

TABLE 41. — BRAIN XVII.

Female. Age, 40 years. Death from pneumonia. In hardening fluid 21 hours after death. $2\frac{1}{2}\%$ bichromate of potash plus one-sixth its volume of 95% alcohol.

TIME — DAYS.	PERCENTAGE INCREASE. ENCEPHALON.
1	6.6 (c.)
60	12.5
73	(c.)
309	12.6
511	14.2

TABLE 42. — BRAIN XIV.

Female. Age, 65 years. In hardening fluid 24 hours after death. $2\frac{1}{2}\%$ bichromate of potash plus $\frac{1}{5}$ its volume of 95% alcohol. Encephalon subdivided except the cerebellum.

TIME. — DAYS.	PERCENTAGE INCREASE.		
	HEMISPHERES.	CEREBELLUM.	STEM.
2.9	15.9	16.1	12.7
5.	(c.)	(c.)	(c.)
30.	21.6	—	16.6
69.	19.9	16.0	14.7
91.	(c.)	(c.)	(c.)
327.	17.6	12.3	11.8
529.	20.0	18.8	17.4

This table, as compared with the preceding table, shows the greater increase in weight where the brain has been subdivided.

TABLE 43. — BRAIN IX.

Male. Age, 57 years. In hardening fluid 10 hours after death. $2\frac{1}{2}\%$ bichromate of potash plus $\frac{1}{2}\%$ copper sulphate, at 35°C . Encephalon completely subdivided.

TIME. — DAYS.	PERCENTAGE INCREASE.		
	HEMISPHERES.	CEREBELLUM.	STEM.
0.8	8.9	15.8	6.9
2.7	13.6	21.3	11.2
7.8	17.2	—	15.1
11.	19.0 (c.)	—	15.1 (c.)
75.	18.7	23.7	15.8
98	(c.)	(c.)	—
323.	18.7	20.7	—
535.	20.3	25.6	17.3

Here, as in the solution with 1% sodium sulphate, the reaction of the cerebellum is peculiar in that it increases in weight decidedly more than the other subdivisions.

TABLE 44. — BRAIN II.

Male. Age, 45 years. Death from Aneurism of the Aorta. In hardening fluid 20 hours after death. In 96% alcohol. Encephalon completely subdivided.

TIME. — DAYS.	PERCENTAGE DECREASE.		
	HEMISPHERES.	CEREBELLUM.	STEM.
0.9	4.0	13.9	14.9
2.9	15.9	21.0	16.3
4.7	19.1	27.4	23.2
6.9	23.4	27.3	24.8
12.7	25.6 (c.)	—	27.3 (c.)
28.8	30.4	—	31.9
91.	30.8	—	31.1
344.	31.4	—	31.5
547.	29.0	28.6	29.9

TABLE 45. — BRAIN VII.

Male. In hardening fluid 24 hours after death. Specimen soft. In 96% alcohol. Cerebral hemispheres alone used.

TIME. — DAYS.	PERCENTAGE DECREASE.
	HEMISPHERES.
1	6.9
3.05	18.2
9.2	25.4
30.2	33.7
87.	34.6
338.	35.7
538.	34.4

TABLE 46. — BRAIN XIII.

Male (negro). Age, 60 years. In hardening fluid 20 hours after death. In 50% alcohol. One cerebral and one cerebellar hemisphere.

TIME. — DAYS.	PERCENTAGE CHANGE.	
	HEMISPHERE.	CEREBELLUM.
1.	+ 10.5	+ 8.2
3.8	+ 7.2	+ 0.6
30.	+ 2.7	— 5.4
92.	+ 0.7	— 6.2
		— 9.6
530.	+ 0.09	— 6.2

If from the foregoing we tabulate the subdivisions of the human brain according to the degree to which they changed in weight, it is as follows:—The subdivision which changed most is marked 1; that which changed least, 3.

TABLE 47.

BRAIN.	SOLUTION.	ORDER OF CHANGE IN WEIGHT.		
		CERE-BELLUM.	STEM.	HEMI-SPHERES.
X	= 2½% bichromate of potash . . .	1	3	2
III	= 2½% bichromate of potash plus 1% sodium sulphate	1	2	3
XIII	= 8% bichromate of potash . . .	2	1	3
IX	= 2½% bichromate of potash plus ½% copper sulphate	1	2	3
II	96% alcohol (decrease)	3	1	2

It must always be remembered that in the case of the human brain, there is one more condition than in that of the sheep. For while these latter are killed when in full health, the human subjects die after more or less prolonged illness, and it has yet to be demonstrated, that the various forms of disease are without influence on this reaction. It remains to be shown how the sheep's brains will react when tested in this same way.

As might be expected, the brains of the newborn react less vigorously to these reagents.

TABLE 48.

Entire encephala of new-born males. Change after 30 days in the hardening solution.

BRAIN.	SOLUTION.	PERCENTAGE CHANGE.
1	2% Bichromate of Potash	+ 25.6
2	2½% Bichromate of Potash	+ 10.6
3	91% Alcohol	— 13.0

In young individuals, both the supporting tissues and the medullary substances are less developed than in the adult, and

this is probably the main reason for the smaller increase in bichromate of potash and the smaller decrease in alcohol.

In closing this paper it need only be pointed out, that in the further handling of hardened tissues many reagents are employed which produce changes in the weight and volume ; and that before we can begin a number of investigations depending on the size and form of the elements, these changes must either be brought under control or eliminated.

Literature.—Here and there in the older literature are many isolated observations on the disturbances caused in animal tissues by the action of reagents on them. In 1891 we gave an account of the changes produced in some of the cranial nerves by the process of hardening and mounting (4), and while this present paper was preparing for the press, Mr. P. A. Fish has published his valuable observations on brain preservation (6).

In addition we may call attention to the observations of Brunetti (2), Hofmeister (3), Sehrwald (1), Van Gieson (5), and Kaiserling and Germer (7). The above observations are directly in line with those by the authors last mentioned, although the technique and material employed in this investigation is quite different from that used by them.

UNIVERSITY OF CHICAGO,
November 14, 1893.

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SYNCÆLIDIUM PELLUCIDUM, A NEW MARINE TRICLAD.

WILLIAM MORTON WHEELER.

THE Triclad which I propose to describe in the following pages was found in the gill-books of *Limulus polyphemus*. My material was collected and in part studied at the Marine Biological Laboratory at Wood's Holl, Mass., during August, 1892. I gladly seize this opportunity of thanking the director, Professor C. O. Whitman, for the many privileges which I have enjoyed at the laboratory, and for his continued interest in my work. I am also indebted to Professor J. S. Kingsley, who has kindly helped me to some of the literature on our native Dendrocœla.

THE TRICLADS PARASITIC ON LIMULUS.

Girard ('50) first gave the following brief description of the common Triclad parasite of *Limulus*:—

“*Vortex candida*, Girard. Body elongated, tapering away towards the posterior extremity;¹ head rounded; sides entire; almost transparent, of a pale rose color. From Chelsea beach,

¹ Girard evidently mistakes the head for the tail end, a blunder into which Ryder has also fallen.

found attached to the Horse-shoe Crab. Length, a quarter of an inch."

The generic position of this species was regarded as doubtful, "the genus *Vortex* not being as yet well circumscribed."

A year later Leidy ('51) gave the following description of a planarian from *Limulus*:—

"PLANARIA, Müller.

Subgenus nov. Bdelloura.¹

Characters same as Planaria, without tentaculæ, and the posterior extremity of the body separated by a constriction serving as a disc of attachment.

5. *BDELLOURA PARASITICA*, *n. sp.*

Body milk-white, with a faint yellowish intestine showing through the translucent integument, smooth, thin, lanceolate, or spatulate; anteriorly narrowed, obtuse; lateral margins, thin, undulating; constricted portion posteriorly truncated, nearly as broad as the middle of the body. Eyes two, reniform. Œsophagus simple, cylindrical, campanulate when protruded.

Length from 3 to 10 lines; breadth 2-5 to $2\frac{3}{4}$ lines. The longest may contract to 6 lines by $3\frac{1}{2}$ lines.

Habitation.—Parasitic upon the King Crab, *Polyphemus occidentalis*, Lam."

The year following, Girard ('52), while remarking on *Fovia*, says: "*Vortex candida*, described at the same time, has been described since by Dr. Leidy under the name of *Bdelloura parasitica*. The genus *Bdelloura* I adopt, but the species must retain its prior name and be called *B. candida*, Girard."

Notwithstanding this identification of *B. parasitica* with *Vortex candida*, Stimpson in his *Prodromus* ('58) retained Leidy's specific name.

These early descriptions were unknown to v. Graff, who in 1879 again described a Triclad from *Limulus* as *Planaria*

¹ βδελλα, a leech; ουρα, tail; because the animal adheres by the tail like a leech.

limuli. v. Graff's paper is a brief but valuable anatomical contribution, to which I shall have occasion to refer again.

It is my opinion that there are at least three distinct species of Triclad to be found on *Limulus*. All the descriptions to which I have alluded are, I believe, referable to the commonest and largest of these species, and for it I shall retain the name *Bdelloura candida*, following the example of Verrill ('73, p. 634). Another species, which I propose to call *Bdelloura propinqua*, is about 8 mm. long, being about half as large as the full-grown *B. candida*. A third species, still smaller (only 2-3 mm. long and .3-.4 mm. broad when fully expanded), differs considerably from the species of *Bdelloura*, so that I have concluded to erect a new genus for its accommodation—the genus *Syncælidium*.¹ When not otherwise stated, the description in the following paper applies to this last species, to which I give the specific name *pellucidum*.

The existence of more than one species of planarian on *Limulus* was first suspected by Ryder ('82^a). He inferred that there might be three species, from three well-marked types of egg-capsules which he found on the gills. That he saw one of the species of *Bdelloura* (probably a half-grown *candida*) is shown by his Fig. 8. His Fig. 10 is undoubtedly taken from a specimen of *Syncælidium pellucidum*, as is shown by the fusion of the posterior gut-rami. In concluding his article, Ryder says: "I do not propose to name the species, as these supposed distinct life-histories may, after all our endeavors to separate them, be only phases of the same thing."

S. pellucidum was also seen by Gissler ('82), as is evident from his Fig. 1. He regarded it as the young of *B. candida*, an error into which he need not have fallen, had he carefully examined the reproductive organs. He seems, however, to have had no clear conception of these organs in Triclads, for he designates the two uteri as sexual glands in his Fig. 1, and in the text remarks: "From analogy I infer the latter to be the male organs, the female glands having escaped my obser-

¹ From *συν*, together, and *κοιλιδιον*, a little intestine, in allusion to the confluence of the posterior gut-rami.

vation." That *Synœlidium* cannot be the young of *B. candida* is at once apparent from the fact that the former species, when fully grown and sexually mature, is considerably smaller than the latter species at the time of hatching!

The appearance of *S. pellucidum*, when resting fully extended on a dark background, is reproduced in Fig. 2 under a low magnification. In the middle the sides of the body are parallel, but towards either end they converge uniformly. The intestine shows through the pellucid body-wall as a flesh-colored mass. Anteriorly the brain is easily seen as a pair of transparent swellings, which form haloes around the two black eyes. Other structures which may be seen with a low power are the pharynx, penis, and the two rosette-like uteri.

Like the species of *Bdelloura*, *S. pellucidum* is very active in its movements, creeping with a steady, rapid motion over smooth surfaces, or swimming at the surface of the water after the manner of the fresh-water Triclad and Pulmonate Mollusca. Rapid movement appears to be very common in the marine Triclads; Lang ('81^b) mentions it in his description of *Gunda segmentata*, and I have observed it in a pretty little *Fovia* which occurs sparingly under the stones between tides at Wood's Holl, but which my friend, Dr. E. O. Jordan, collected for me in great numbers on Monhegan Island, Me.

Synœlidium occurs only between the leaves of the gill-books of the *Limulus*, never migrating to the bases of the cephalothoracic legs like the young and sexually immature *Bdelloura*.

THE ANATOMY OF SYNCÆLIDIUM.

1. *Integument.*

The rather dense cilia covering the whole body are somewhat longer on the ventral than on the dorsal surface (Fig. 10); measuring on the former $.4\ \mu$, on the latter $.2\ \mu$ — $.3\ \mu$. They are borne by a compact membrane (z) which stains quite deeply in the various carmines. In cross-section it appears to be homogeneous and somewhat thicker on the ventral ($.3\ \mu$) than on the dorsal surface ($.25\ \mu$). No nuclei could be detected in this membrane, but when the planarian was dried

over a flame—either in the fresh state or after long preservation in alcohol—a beautiful net-work appeared on its surface (Fig. 6). The net itself is dark-colored, but the polygonal spaces enclosed by its meshes are much paler. In sections, when the knife skims off the surface of the body, the same structure is apparent, but with the tones of color reversed: the net being colorless while the spaces take the stain. The same structure is also visible in sections of large specimens of *B. candida*. The ciliated membrane so closely resembles the pharyngeal epithelium of fresh-water Tricladæ after treatment with silver nitrate (see the Figs. of Woodworth, '91, and Chichkoff, '92), that I take the net-work to be an indication of cellular structure. Each polygonal area probably represents a cell. The lack of any traces of nuclei in these areas puzzled me at first, but I believe that they are to be found just beneath the longitudinal muscles (Fig. 10 *hy*). Occasional processes from these hypodermal cells may be seen extending out between the muscle-strands and joining the plaque-like polygonal pieces of the ciliated membrane. Sometimes the nuclei lie between the muscle-strands or even nearer the surface. From these conditions I conclude that the hypodermal cells of *Syncalidium* are mushroom-shaped, the cell-body being represented by a polygonal ciliated umbrellar portion with a stem in which the nucleus is lodged and which extends in some distance below the surface and between the muscle-strands. The portion of the hypodermal cells which I have called the ciliated membrane—and which, if my interpretation be correct, is really no membrane at all—is liable to separate from the underlying muscle layer in specimens killed in hot sea water (Fig. 10).

2. Musculature.

Immediately beneath the ciliated portion of the hypodermis—providing I have not overlooked some structure corresponding to the basilar membrane of the fresh-water Tricladæ—lies a very delicate layer of transverse muscles, which may be detected in thin tangential sections. The smooth fibres, of which this layer is composed, vary somewhat in

width. Between them lie minute and much elongated nuclei, which, I believe, belong to them, notwithstanding the almost unanimous assertion of writers that the adult muscles of the *Dendrocoela* are enucleate. As I have made no macerations of these muscles, I can make no definite statements in regard to their method of termination and attachment. They do not pursue a very long course as discrete fibres, but appear to anastomose at intervals, thus forming a net with very much elongated meshes.

Beneath the delicate transverse muscles lies a very thick layer of longitudinal muscles (Fig. 10 *ml*), which consist of distinct and quite regular bundles. These extend from one end of the body to the other, without or with very rare anastomoses. They appear as clear strands in stained preparations. On an average they are about $1.3\ \mu$ in breadth, with a dorso-ventral diameter of $3\ \mu$. Measurements, of course, vary greatly according to the state of contraction of the fibres. In many of my preparations a peculiar striated or lamellated appearance may be observed in the muscular substance (Fig. 10). It seems to be due to an alternation of dense and stainable with an unstainable and more liquid plasma. The lamellæ are usually arranged parallel to the surface of the body or at a slight angle to it. I fail to find any nuclei in the longitudinal muscles; the nuclei which lie between the strands, often in very regular rows, and immediately beneath them, belong, as I have said, to the hypodermis.

In *B. candida* the longitudinal muscles are very poorly developed as compared with those of *S. pellucidum*. The dorsoventral muscles, so well developed in the former species, are so difficult to resolve in the latter that I am not sure that I have seen them. Owing to its small size, *Synœlidium* is not a very favorable object for the study of the various muscle-layers of the pharynx and penis.

3. *Parenchyma*.

So much space in the body of *Synœlidium* is taken up by the longitudinal muscles and viscera, that the parenchyma — abundant in many other Triclad — is here very much reduced

and difficult to analyze. A complete lack of pigment adds to the difficulty of locating the cells peculiar to this interesting tissue.¹

On the other hand the glands are very easily studied. In specimens stained with Weigert's picrocarmine and mounted in glycerine they take on a deep yellow or orange color, in Czokor's alum cochineal they are colored a dull brick red. In both cases they contrast strongly with the surrounding tissue.

At first sight the glands appear to have a very diffuse distribution, but closer study enables one to separate them into the following groups (Fig. 7) :

1. There is a narrow zone of slime-glands completely surrounding the body just within the lower ventral edge. This zone, which appears to be common to many other Triclad's (*vide* Lang, '81b, Iijima, '84, Woodworth, '91), is broad in the head region, distinctly narrower in the tail, and very narrow along either side of the middle of the body.

2. A large group of slime-glands in the head region. These have an anterior trend and open on the dorsal surface. In the region of the eyes they separate into three bundles, one of which passes between the eyes and over the brain, while the two others pass laterally to the eyes. The three bundles again intermingle in the præocular region.

3. A somewhat smaller group of slime-glands at the opposite end of the body with a posterior trend and openings on the dorsal surface.

4. A cluster of glands corresponding to the salivary glands of Iijima ('84), and Chichkoff ('92). They are found in the parenchyma just in front and on either side of the pharynx, to which they converge, and into which they open near its tip.

5. A cluster of glands surrounding the vaginal opening in the genital atrium (Fig. 7 and Fig. 4gg1).

So far as their reaction to the stains is concerned, the glands of these five groups are identical in *Syncalidium*. In *B. candida*, however, the salivary glands have a specific reaction : they take on a very intense blue stain with alum

¹ In this lack of pigment *Syncalidium* resembles the fresh water *Dendrocalum lacteum*.

cochineal, while the glands of the other categories stain dull brick red like their homologues in *Syncalidium*. So deep is the stain that full-grown specimens of *B. candida* mounted *in toto* in clove-oil or balsam show the salivary glands as a mass of nodulated fibres radiating from the pharynx into the body. v. Graff ('79) supposed that the lesions in the gill-lamellæ of *Limulus* were produced by some chitin-solvent in the secretion of the slime-glands of *Bdelloura*. May not the differential stain of the salivary glands in this form point to the pharynx as the organ which emits this chitin-solvent?

v. Graff describes in *Bdelloura* two acinose glands, opening one on either side into the pharynx. I fail to find these unless they be the "salivary glands." In that case the word acinose ("traubenförmig") is certainly ill-chosen. Moreover, these glands are not separable into two clusters in my specimens.

In regard to the slime-ducts and their openings in Tricladæ, I am unable to share the view recently advanced by Chichkoff ('92). According to this author (p. 485) there are no determinate ducts or orifices; in order to reach the surface of the body the mucus makes a way for itself through the parenchyma and hypodermis. "L'excrétion," he says, "se fait sur différents points, suivant les besoins; selon que l'excitation se produit sur la face dorsale ou ventrale, la substance se dirige vers l'une ou vers l'autre, pour être expulsée. Ce que Iijima désigne dans ses figures sous le terme de 'Ausmündungsstelle der Schleimdrüsen' n'est rien d'autre que des traînées de mucosité sur le point de s'échapper du dehors. Leur présence, observée presque toujours sur les coupes, est due uniquement aux conditions dans lesquelles l'animal a été fixé." And again at p. 486-487 he adds: "Les 'Ausmündungsstellen' disparaissent complètement, si l'on fixe l'animal en évitant la présence de la substance muqueuse dans le parenchyme, autrement dit, si on lui donne le temps d'expulser tout ce que ses glandes ont sécrété, du moment où la spatule l'a pris."

Granting that all preceding observers have mistaken the products of secretion for portions of the glands, does it follow that the slime-glands have no determinate ducts and openings? Not in the least. Such delicate structures as these ducts

must be, would, of course, be very difficult to resolve in specimens killed just after the momentary supply of mucus had been exhausted. In *Syncælidium* it is no difficult task to trace the irregular slime-ducts, or at any rate the "trainées de mucosité," to the spaces between the polygonal areas of the ciliated membrane. I believe the little widenings occurring at intervals in these spaces are the openings of the ducts. I cannot, therefore, agree with Chichkoff, and must hold to the views of Lang, Iijima and Woodworth, though I am not prepared to agree with the last mentioned observer in homologizing the slime-glands with the "Stäbchenstrassen" of the Rhabdocæles.

4. Digestive Tract.

The pharynx does not differ in its form or function from that of other Triclad s so far as I can observe. It is a thick-walled muscular tube (Fig. 10 *ph*) contained in a special chamber and opening into the trifid gut.

The gut, or intestine, deviates somewhat from the Triclad type (Figs. 3 and 4 *coe*). The unpaired anteriorly directed ramus does not extend up to the brain, much less beyond it as in *Gunda* and *Phagocata*. Its lateral diverticula are few in number (five to seven on a side) compared with such forms as *Dendrocælum lacteum* or even *Bdelloura candida*. The diverticula show little tendency to subdivide, except at their tips, where they are sometimes bi- or even trifurcate. The two posterior gut-rami pass back, one on either side of the pharyngeal chamber, and give off a few stunted diverticula, which have even a less tendency to subdivide than the diverticula of the anterior ramus. Behind the genital atrium the two rami converge and fuse. From their point of union an unpaired stem with a few diverticula on either side extends posteriorly a short distance. The diverticula usually alternate so that the stem has a zigzag appearance.

This curious fusion of the posterior rami is constant in all the specimens of *Syncælidium* which I have examined (about 75 in number) with two exceptions. These were just hatched young (.5-.6 mm. long). One of these (Fig. 1) shows the

rami separate, that of the right side being considerably longer than that of the left. I conjecture that in the fusion, which must take place very soon after hatching, the tip of the shorter ramus unites with its fellow in such a way that the whole tip of the primitively longer ramus is left as the unpaired stem of the adult.

The fused condition of the posterior rami of *Syncælidium* was described and figured by Ryder ('82^a, Fig. 10). v. Graff says of *B. candida*: "Die beiden hinteren Darmschenkel sind in der Jugend getrennt, bei erwachsenen Thieren aber (immer?) durch eine Queranastomose verbunden." This condition I have also noticed in three adult specimens of *B. candida*, but the fusion is of a very different nature from that obtaining in *Syncælidium*. In the former species the posterior rami are of equal length and extend into the tail. In the cases which I have examined, the fusion occurs between two mesial diverticula, thus forming a connection between the rami, like the horizontal bar which joins the two upright pieces in the letter H. This vinculum does not occur in the young or half-grown specimens of *B. candida*. In *B. propinqua* I have seen no trace of it.

Only one other Triclad is known to me which exhibits a union of the posterior rami — a species of *Dendrocoelum* (*D. nausicaæ*) from Corfu and Cephalonia, long since described by Oscar Schmidt ('61). His Fig. 1, Pl. II, shows that the union is brought about by a confluence of a pair of mesial diverticula, and not by a fusion of the rami themselves. The species is interesting as presenting a condition intermediate between *Syncælidium* and *Bdelloura*, for one of the posterior rami is distinctly shorter than the other.¹

Histologically, the gut of *Syncælidium* does not differ from that of other Triclads as described by Lang, Iijima, and Chichkoff.

¹ In Boas' Text-book of Zoology ('90, p. 150), there is a figure of *Dendrocoelum lacteum* attributed to Oscar Schmidt, and showing several anastomoses between the posterior gut-rami. In N. American specimens which I regard as belonging to this species I can detect no such conditions, the two posterior rami being quite separate.

5. *Excretory System.*

In hardened specimens and in sections I have looked for the water-vascular system in vain, but in the living animal more or less extensive portions of it were readily detected when just the right amount of compression was applied. It consists of a rather stout and somewhat zigzag main trunk on either side of the body (Fig. 4 *tex*), running just lateral to or immediately over the tips of the gut-diverticula. The trunks arise near the anterior end of the body and fade away in the caudal region. Into the main trunks open at very irregular intervals, and often by twos and threes, jagged and ramified branches. No flame-cells were seen in the main trunks, but at intervals in slight dilatations of the larger branches (Fig. 11 *wf*) the play of the flagella could be watched for minutes at a time. The movements of the flagella are very rapid, and resemble an endless chain moving behind a slit-shaped opening in a direction parallel to the long axis of the slit. I have not seen the funnels figured by Lang and Chichkoff. The two main trunks are connected with each other at the posterior end of the body by means of anastomosing smaller branches. A similar connection at the anterior end could not be made out. I do not lay much stress on this negative observation, since Iijima figures an anterior connection between the main trunks in the young *Dendrocoelum lacteum* (Pl. XX, Fig. 2), although he seems not to have found a posterior connection. Chichkoff also gives an excellent figure of the anterior anatomoses in the same species (Pl. VIII, Fig. 38).

The pharyngeal excretory system so beautifully figured by Chichkoff (Pl. VIII, Fig. 41) was not seen in *Syncoelidium*. Nor have I seen anything which might be interpreted as an opening of the water vascular system to the exterior, either on the dorsal surface (dorsal pores of Iijima) or in the pharynx, where the occurrence of an opening is suspected by Chichkoff.

6. *Nervous System.*

The great transparency of *Synœlidium* makes it a very favorable object for the study of the nervous system. The brain and main nerve-trunks may be readily seen in the living animal, but this method is insufficient for a study of details. It is, however, only necessary to stain with alum cochineal, extract as much of the stain as possible with water, dehydrate and mount directly from absolute alcohol in gum sandarac to obtain a diagrammatically clear picture of all but the very finest details of the nervous system.¹ The nerves stand out as white lines on a darker background. Fig. 3 is a camera-drawing from such a preparation, the color of the nervous and non-nervous tissue being reversed for the sake of greater clearness.

The two longitudinal trunks (Fig. 3 *vnv*) gradually widen anteriorly and unite to form the brain (*br*) which, like the brain of *Gunda* (Lang, '81^a) and other Triclad, may be divided into an anterior and superior, or sensory, and a posterior and inferior, or motor portion. From the former portion arise, on either side of the median line, three sensory (?) nerve trunks, which run forward and outward. The two inner trunks (*an*¹, *an*²) break up into a plexus before they reach the marginal nerve (*mnv*) which runs around the entire periphery of the body. The third nerve on either side remains undivided. The marginal nerve is very poorly developed between the tips of the mesial branches arising from the first sensory nerve on either side. Behind the brain each longitudinal nerve is connected with the marginal nerve of its own side by means of a very regular series of lateral nerves (*lnv*). I am not sure that the number of these lateral nerves is always constant; in the specimen from which the figure was taken there were twenty-one, exclusive of the third and undivided sensory (?) trunk. Twenty of the lateral nerves on either side are connected by transverse nerves (*trnv*), which bridge the space between the longitudinal trunks. Their points of insertion in the longi-

¹ Sandarac is preferable to balsam on account of its lower refractive index.

tudinal trunks correspond exactly to the points of exit for the lateral nerves. At the posterior end of the body, where the longitudinal nerves unite, a small plexus of somewhat variable character connects their fused ends with the marginal nerve.

This I take to be the typical arrangement of the main nerve-trunks. In a few cases, however, I observed a marked deviation from this schema; some of the transverse nerves in the retro-pharyngeal region had broken up into a rude plexus, much like the plexus which occurs in fresh-water Triclad's throughout the whole area between the longitudinal cords.

The marginal nerve has a clearly defined outline along its inner border, but along the outer edge it is quite irregular, and breaks up into an indistinct plexus of smaller fibres not easily resolved in *Synœlidium*. In specimens of *B. candida*, which had been hardened in a much flattened condition, I found no difficulty in tracing this plexus for some distance externally to the marginal nerve as a pretty net-work of fibres with rather uniform meshes. I have no means of telling how extensive this plexus may be. A long series of experiments with methylene blue, undertaken for the purpose of establishing this point, failed completely. I am, however, inclined to believe with Woodworth, that the plexus, of which the marginal nerve is only a condensation, extends through the longitudinal muscles, especially in *Synœlidium*, where this system is so powerfully developed.

In young specimens, a ring-nerve may be seen in the walls of the œsophagus (Fig. 3, *phr*) connected with two lateral pharyngeal nerves (*phl*). The innervation of the pharynx thus resembles that of *Gunda* as described by Lang ('81^a, '81^b). I have been unable to make out a connection between the lateral pharyngeal nerves and the longitudinal trunks, but I doubt not that such a connection exists.

On the histology of the nervous system I have little to add to the accounts of other writers (Iijima, Lang, and Chichkoff). In *B. candida*, where the histological elements are more robust, I have found spindle-shaped ganglion cells in the marginal nerve similar to the ganglion cells which occur in the longitudinal nerves of the same and other Triclad's. These cells

were not seen in the marginal nerve of *Syncalidium*. In regard to the brain, I must dissent from Woodworth, who regards the "Substanzinseln" as consisting of connective tissue, and the mass of closely-packed cells with large nuclei surrounding the fibrous portion of the brain as mesenchymatous. In *Syncalidium* it is possible to entertain some doubt in regard to the exact nature of these cells, because of their small size; but in *B. candida*, both the "Substanzinseln" and the enveloping cells are undoubtedly ganglionic. It is even possible in this form to trace the single process of each ganglion cell into the felted mass of brain-fibres.

In *B. propinqua*, the brain with its ganglionic covering is separated from the surrounding tissues by a layer of peculiar fibrous tissue. In specimens mounted *in toto*, this layer appears as a pale halo surrounding the brain, and considerably increasing its apparent size.

The eyes of *Bdelloura* and *Syncalidium*, always two in number, consist of oval refractive bodies set in pigment-cups, the latter being confined to the inner and lower surfaces of the bodies. The eyes lie, one on either "Substanzinsel," and appear to be innervated by very short fibres from this region of the brain.

7. Male Reproductive Organs.

The testicular follicles (Fig. 4 *ts*) are a series of oval, or, very rarely, lobed bodies which lie on either side of the body between the successive gut diverticula. They extend from the second or fourth diverticulum of the anterior ramus to the second or third diverticulum of the unpaired stem. They vary considerably in number; in twenty specimens in which they were counted, there were 8-20 on either side of the body, the average number being 14. In the same individual the number on both sides is rarely equal; one side usually exceeds the other by from one to four follicles.

In *B. candida* the testicular sacs are much more numerous (60-100 on either side), and each sac is relatively very much smaller. Moreover, they lie outside the tips of the gut diverticula rather than between them. In *B. propinqua* the testicular

sacs are of about the same relative size as in *B. candida*, but nearly twice as numerous; in onespecimen I counted 170 on one side of the body and this is probably below rather than above the average number for this species. Here, too, the sacs extend in between the gut diverticula as compact rows, especially in the anterior ramus.

In sections of *Syncalidium* the wall of each follicle consists of a single layer of cuboidal cells with large, round nuclei (Fig. 10 *tsf*). Towards the lower inner edge of the sac the cells flatten out, and are continued into a thin string-like duct, which I have succeeded in tracing for some distance towards the vasa deferentia. I have no doubt that Woodworth and Chichkoff are correct in regarding these ducts as preformed and determinate paths by which the spermatozoa reach the vasa deferentia.

The testicular follicles are normally packed full of spermatozoa in various stages of maturescence. With alum cochineal the fully developed male elements take an intense indigo blue tint, which contrasts strongly with the purple color of the follicular cells and younger spermatozoa. This intense blue is very probably an expression of their powerfully cyanophilous character, and is of interest in connection with the recent researches of Auerbach ('91a, '91b) and Watasé ('92).

The vasa deferentia (Fig. 4 and Fig. 10 *vd*) lie on either side of the pharyngeal chamber, and extend from the region of the fourth or fifth diverticulum of the anterior gut-ramus to the keg-shaped penis. In mature specimens they are always distended with spermatozoa and sharply marked off from the surrounding tissues. Their anterior ends are thread-like, but posteriorly they widen out considerably and finally take a short and abrupt turn headward, only to turn back again and run as very delicate tubules into the penis. These delicate ejaculatory ducts widen towards their tips and open by discrete ostia very near the orifice of the penis. There is, therefore, no unpaired ejaculatory duct in *Syncalidium* as in many other Triclad.

The penis lies in the genital atrium (Fig. 3 *gat*), which is provided with a small opening on the ventral surface of the body.

8. Female Reproductive Organs.

The ovaries are a pair of round translucent bodies, one on either side of the anterior gut-ramus, between its first and second, or second and third diverticula (Fig. 4 *ov*). They appear to be simple sacs consisting of small, flattened cells enveloping the maturing ova. I can detect nothing which may be regarded as a parovarium in *Synœlidium*.

The oviducts (Fig. 4 *ovd*) leave the posterior outer surfaces of the ovaries as thin tubes. At first they run a little within and then along the dorsal surface of the longitudinal nerve trunks (Fig. 10 *ovd*) till they reach a point in front of the confluence of the posterior gut-rami. Here they converge and unite in the median line to form a very short vagina, which opens on the posterior wall of the genital atrium (Fig. 4 *vg*). The vagina is supplied with a number of slime-glands (*ggl*), which are undoubtedly homologous with the glands figured in a similar situation by Iijima for *Planaria polychroa* ('84, Fig. 5, Pl. XXI *dr*), and *Dendrocœlum lacteum* (Fig. 1, Pl. XXI *edr*), and by Lang for *Gunda segmentata* ('81b, Fig. 56, Pl. XIV).

The vitellaria are granular cell-cords which in the sexually mature *Synœlidium* fill all the available interstices between the gut diverticula medially to the testicular sacs. Their general arrangement may be gleaned from Fig. 4 *vt*; their cytological structure from Fig. 10 *vt*. In sections the cell-cords are found attached to the walls of the oviducts, but on the method of their opening into the latter I have made no observations.

In his account of the anatomy of *B. candida* ('79) v. Graff makes the following statement: "Auffallend erscheint dagegen die Duplicität des Uterus, der jederseits zwischen 7. und 8. Darmmast (von hinten gezählt), durch je eine besondere Mündung rechts und links vom hinteren Ende des Schlundrohrs sich nach aussen öffnet." Before I had seen this statement I was surprised to find two uteri in *Synœlidium* (Fig. 4 *u*). They are spheroidal bodies conspicuous in the living animal as translucent foam-like masses on either side of the genital atrium and forming apparent interruptions in the posterior gut-

rami. The center of each mass is usually, although by no means always, filled with a more opaque substance (*sp*) immediately surrounding which there is a pale halo (*us*). In carefully stained specimens each uterus is seen to possess an independent opening on the ventral surface (*ou*). The relations of these openings to the longitudinal nerve-cords are seen in Fig. 3 *ou*. The apertures occupy the posterior inner edges of uteri. In preserved specimens the apertures themselves are very small, but there is a pale enucleate halo surrounding each, so that they may be readily found.

In sagittal section (Fig. 8) the uterus is seen to take up nearly the entire thickness of the body. Externally its wall is sharply defined, but internally it is very ragged, at least in certain stages of its physiological activity, the single layer of cells composing it being frayed out at their inner ends. The nuclei of these cells (*x*) are few in number and stain very faintly. The mass in the center (*sp*), which shows as an opaque body in specimens studied *in toto*, is a compacted ball of spermatozoa, and the pale halo surrounding it (in the section Fig. 8 represented by two fragments only, *us*) is a glairy coagulum which usually envelops the mass of spermatozoa, and which I take to be the secretion of the cells of the uterine wall. The opening of the uterus is shown in section at *ou*. The walls of the delicate lumen, which appear to consist of a chitinous substance, are very thick and concentrically striated. A small nodule (*vl*), perhaps a kind of valve, seems to occlude the lumen. Then follows a more dilated cavity communicating with the cavity of the uterus proper.

In *B. candida* the conditions are similar, but more easily studied on account of the much greater size of this Triclad. The duct runs to the exterior from the anterior instead of the posterior inner surface of the uterine sac. In section (Fig. 9) the epithelial character of the wall is easily studied. It agrees in all essential respects with the uterine epithelium of the fresh-water forms. The duct has a very distinct lumen (*ou*) which is ciliated up to the valve (*vl*). There is also a slightly dilated portion connecting the duct with the uterine cavity proper as in *Synœlidium*. Contrary to v. Graff's

supposition, I find no connection between the oviducts and the uteri.

Bdelloura and *Synœlidium* appear to be the only Triclad s which have two uteri and a distinct duct and opening for each uterus on the surface of the body.¹ The gap between these ectoparasitic planarians on the one hand and the fresh-water and land planarians on the other, is partially bridged by a series of forms representing the gradual emancipation of the oviducal opening from the duct of the uterus (vagina of authors).² In the land Triclad s and in *Gunda lobata* (O. Schmidt) the oviducts unite with the hind portion of the uterus; in *Haga plebeja* they open into the lower end of the uterus; in *Gunda segmentata* and *G. ulvae* the unpaired portion of the oviduct opens into the duct of the uterus; in the common fresh-water species it can hardly be said to open into the duct of the uterus, but has a separate opening into the genital atrium. In all these forms, however, the duct of the single uterus opens directly into the genital atrium; in *Bdelloura* and *Synœlidium* the ducts of the *two* uteri appear to have no connection with the oviducts and are entirely removed from the genital atrium. I fail to find any traces of yolk cells or ova in the uteri and I have seen nothing to indicate that the egg-capsules are formed in these organs. Spermatozoa are nearly always present in considerable quantity. In a specimen of *B. candida* I found spermatozoa in either oviduct for some distance up towards the ovaries. In a specimen of *Synœlidium* I found an egg about to be discharged into the genital atrium. These few observations are calculated to render the vexed question of the function of the uterus in Triclad s still more perplexing. The great variation in the position and shape of the uterus—in other words, its morphological instability—in Triclad s points to considerable variety of function. For the present I am inclined to believe that the uteri of *Synœlidium*

¹ Unless, indeed, this latter peculiarity obtain also in Bergendal's *Uteriporus vulgaris*. I have not seen his description of this form, but I conclude that its single uterus has an opening on the outer surface of the body from a remark in his recent paper ('92 p. 313).

² What I have called the vagina in *Synœlidium* is not, of course, homologous to the vagina of other Triclad s.

and *Bdelloura* have nothing to do with forming the egg-capsule, but function only as receptacula seminis. On this supposition it is difficult to explain the presence of a secretion in the uterus unless it serve as a nutrient medium for prolonging the activity of the spermatozoa. It would seem, either that the duct of the uterus must function as a vagina, the penis being inserted in its orifice during copulation, or that the spermatozoa must be attracted into the uterine cavity. This may perhaps be accomplished by positive chemiotactic properties resident in the secretion of the uterine epithelium. I take this suggestion from Jordan's interesting paper on the spermatophores of the newt ('91); he believes that the spermatozoa of this amphibian may be enticed into the recesses of the cloacal glands by the glandular secretion.

9. *Breeding Habits.*

B. candida, *B. propinqua* and *S. pellucidum* all deposit their egg-capsules on the gill-lamellæ of their host, *Limulus*. The first species seems to show no preference for a particular region of the gill-leaf, but scatters its egg-capsules over the whole surface. *B. propinqua* selects the basal, or proximal region of the leaf, while *Synœlidium* prefers a small area near the edge and just lateral to a small marginal callosity which forms a brown line with the callosities of the adjacent leaves when the gill-book is closed.

The egg-capsule of *Synœlidium* (Fig. 5) is about .75 mm. long, of an oblong shape and somewhat compressed. It is attached by a slender pedicel .5 mm. in length, in such a way that one of the flattened sides of the capsule is applied to the surface of the gill-leaf. Usually the capsules are arranged with their long axes parallel to one another in a little cluster near the marginal callosity. The chitinous wall of the capsule is thin and transparent, but grows thicker towards the poles. Through it the two opaque white eggs or larvæ may be distinctly seen. I have never found more than two eggs in a capsule.

Many of the capsules bear at their outer ends one or more of the deep blue thecæ of an infusorium (Fig. 5). These

were regarded by Gissler ('82) as pneumatic tubes, but Ryder ('82b) showed that they were the thecæ of "Protozoa of the genus *Epistylis* or *Zoothamnion*."

Both Ryder ('82a) and Gissler ('82) figure the egg-capsules of *Synœalidium*. After describing the capsules of *Bdelloura*, Ryder says: "The second form, represented in Figs. 5-7, enlarged 16 times, is much smaller, but similar in structural features to the preceding. The capsules measure about $\frac{1}{25}$ of an inch in length and contain usually 2 eggs or embryos. At first the ova occupy each one of the ends of the capsule, as shown in Fig. 5; but after the young worms have developed somewhat, they usually lie alongside of each other lengthwise of the capsule. They frequently change positions, however, at this stage and it sometimes happens that there is but one embryo in a capsule."

Gissler's Fig. 2^b is evidently the capsule of *Synœalidium*, as shown by its size relatively to the infusorial thecæ attached to its summit.

For a description of the egg-capsule of *B. candida* I would refer the reader to the papers of Leidy ('51), v. Graff ('79), Ryder ('82a) and Gissler ('82).

What I take to be the egg-capsule of *B. propinqua*, is considerably smaller than that of the allied *B. candida*, measuring only 1.25 mm. It appears to contain only one ovum, instead of 2-7 as in *B. candida*, but on this point I cannot be positive. I am unable to identify this form of capsule with any of those described by Ryder ('82a).

The three *Limulus*-infesting Tricladæ differ also in their time of breeding. *B. candida* oviposits during May and early June, when the *Limuli* return from the deep water to the sandy beaches to breed. The passage of the Tricladæ from one crab to another must be favored by the prolonged coitus of the latter. *Synœalidium* oviposits in the latter part of July and the early part of August, when the gills are deserted by the half-grown young of *B. candida* for the basal joints of the cephalothoracic appendages. As the *Limuli* have laid their eggs and begin to return to deep water by the first days of July, it is necessary in order to study *Synœalidium* and its

habits, to collect a number of the crabs early in the season and to confine them in a large fish-box or similar receptacle. *B. propinqua* appears to breed at the same time as *Synœlidium*.

10. General Conclusions.

In conclusion two questions on which the foregoing anatomical study of *Synœlidium* has a bearing may be briefly discussed. The first relates to the origin of metamerism, the second to the systematic position of the three Triclad's parasitic on *Limulus*.

A careful study of the strikingly regular anatomical features of *Gunda segmentata* led Lang ('81b) to attempt a derivation of the Hirudinea from Turbellaria-like forms. Such a derivation involves *implicite* the view that metamerism in the Annelida and higher forms is not derived from a process of budding like that observed in such forms as *Microstomum*, but from a condition like that which obtains in *Gunda*, where the testes, vitellaria, gut-diverticula and transverse portions of the nervous system have a segmental arrangement. This view has many attractions and I was inclined to give it great weight when I began my study of *Synœlidium*. Here, too, the nervous system has an arrangement quite as regular as that of *Gunda*, and preliminary observation pointed to a pair of gut-diverticula, a pair of testes and a pair of vitellaria to each transverse and each pair of lateral nerves. But the examination of a great number of specimens of all ages soon convinced me that the gut-diverticula are very variable in number and arrangement, that they are not paired and do not coincide with the lateral nerves. The testes and vitellaria accommodate themselves closely to the gut-diverticula, and vary correlatively. The water vascular system and musculature, of course, showed no signs of metamerism, so that I was left with the nervous system as the only apparently metameric organ in the body of *Synœlidium*. Hence, providing Lang's Fig. 1 is not unduly schematized,—and I am not willing to believe this,—I am forced to admit that *Synœlidium* falls far short of *Gunda* in the metameroid arrangement of its parts. The *Limulus*

parasite is interesting as representing a condition intermediate between *Gunda* and the fresh-water Triclad, a condition in which the nervous system is quite as regular as that of *Gunda*, while the other organs are scarcely more regular than their homologues in the fresh-water species.

The most striking peculiarity of the Triclad parasites of *Limulus* is the duplicity of the uterus and the independent openings on the surface of the body. v. Graff, who first observed this curious character ('79), does not appear to have given it due weight. This character combined with another, *viz.*, the complete absence of rhabdites, seems to me to be of sufficient magnitude to isolate these Triclad as a distinct family — Bdellouridæ — coördinate with the families Planariidæ and Geoplanidæ. Certainly the characters which distinguish this proposed new family from the remaining Triclad are as important as those used in the delimitation of many of the families of Polyclads. As a purely tentative arrangement of the group, subject, of course, to modification in the future, I would propose the following:

Family Bdellouridæ.

Ectoparasitic marine Triclad without auricular folds at the cephalic end; without pigment, except in the two eyes; without rhabdites; with two uteri opening by discrete ostia laterad to the longitudinal nerves; ejaculatory ducts opening separately very near the tip of the penis. Egg-capsules, elliptical or oblong, flattened, attached by a slender pedicel.

* Large species, with typical Triclad gut; the posterior rami united by a fusion of two of the mesial diverticula only in old specimens (always?); anal end of the body widened into a glandular disc; anterior end narrow and tapering to a point when the animal is expanded; ducts at the anterior edges of the uteri; penis acuminate, with a broad base. BDELLOURA, Leidy.

† Testicular sacs small, about 60–100 in number on either side of the body, lying laterad to the gut diverticula; brain relatively small, not contained in a fibrous capsule: Length

when fully grown, 15 mm. Egg-capsule elliptical, length 2.5-4 mm.

B. candida, Girard (*B. parasitica* Leidy; *Planaria limuli* v. Graff).¹

†† Testicular sacs small, about 170 in number, lying on either side of the body, but extending inward a considerable distance between the gut-diverticula, especially in the anterior region of the body; brain enclosed in a fibrous capsule. Length when fully grown, about 8 mm. Egg-capsule elliptical, length 1.25 mm. *B. propinqua* nov. sp.

** Small species, with the posterior rami of the gut uniting soon after hatching and forming an unpaired stem; both ends of the body alike, tapering, when the animal is fully extended. Ducts at the posterior inner surfaces of the uteri. Penis keg-shaped. SYNCCELIDIUM, nov. gen.

† Testicular sacs very large, the average number being 14 on either side of the body; lying between the simple gut-diverticula; brain relatively large, not enclosed in a fibrous capsule. Length, 3 mm. Egg-capsule oblong, length .75 mm. *S. pellucidum*, nov. sp.

THE UNIVERSITY OF CHICAGO,

March 1, 1893.

POSTSCRIPT.

My attention was not called to Verrill's paper on the Marine Planarians of the New England coast (Trans. Conn. Acad., Vol. VIII, January, 1893) till after I had handed in my manuscript for publication. Verrill has anticipated me in founding a family for the reception of *Bdelloura candida*—the only species which he recognizes; but the characters which he assigns to this family are not in all respects satisfactory.

¹ Leidy ('51) describes another species as *Bdelloura rustica*, from Egg Harbor, N. J., where it was found on *Ulva latissima*. I do not include this in my table, because I doubt that it belongs in the genus *Bdelloura*. Equally doubtful is Stimpson's allocation ('58) of *Planaria longiceps* of Dugès ('30) in the same genus.

Thus he omits the double uterus, in my opinion the most distinctive character of the group. The posterior acetabulum, or sucker, must be dropped as a family character, because it is lacking in *Synœlidium*, a form too closely allied to *Bdelloura* to be relegated to any other Triclad family.

In Verrill's anatomical sketch of *Bdelloura candida*, I would criticise a remark on the uterus. At page 123, he says: "Each one (uterus) is connected with the genital duct by a convoluted tube"; and in his Fig. 8, Pl. XLIV, he clearly depicts this structure. When the uteri are much shrunken, their irregular edges may perhaps simulate a convoluted tube, but as Verrill has not studied sections of the Planarian in question, and as I feel quite confident that I have not overlooked this tube leading to the "genital duct," I doubt its existence, at least in *Synœlidium*.

April 2, 1893.

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EXPLANATION OF PLATE VIII.

<i>an</i> ¹ .	First sensory nerve.	<i>sp.</i>	Spermatozoa.
<i>an</i> ² .	Second sensory nerve.	<i>tex.</i>	Main trunk of water vascular system.
<i>br.</i>	Brain.	<i>dvml.</i>	Dorsoventral muscle.
<i>coe.</i>	Gut.	<i>truv.</i>	Transverse nerve.
<i>e.</i>	Eye.	<i>ts.</i>	Testicular sacs.
<i>ggl.</i>	Glands opening into the vagina.	<i>tsf.</i>	Follicular epithelium of testis.
<i>hy.</i>	Hypodermal cells.	<i>u.</i>	Uterus.
<i>lnv.</i>	Lateral nerve.	<i>uep.</i>	Epithelium of the uterus.
<i>ml.</i>	Longitudinal muscle.	<i>us.</i>	Secretion of the uterus.
<i>mnv.</i>	Marginal nerve.	<i>vd.</i>	Vas deferens.
<i>ou.</i>	Ostium of the uterus	<i>vl.</i>	Valve-like nodule in the uterine duct.
<i>ov.</i>	Ovary.	<i>vuv.</i>	Longitudinal nerve.
<i>ovd.</i>	Oviduct.	<i>vt.</i>	Yolk-glands.
<i>p.</i>	Penis.	<i>wf.</i>	Wimperflamme.
<i>ph.</i>	Pharynx.	<i>x.</i>	Nucleus of the uterine epithelium.
<i>phl.</i>	Longitudinal nerve of the pharynx.	<i>z.</i>	Ciliated membrane.
<i>phr.</i>	Ring-nerve of the pharynx.		
<i>sgl.</i>	Slime-glands.		

FIG. 1. Just hatched *Syncalidium*, showing the still separate posterior gut-rami.

FIG. 2. Adult living *Syncalidium* as seen in reflected light on a dark surface ($\times 10$).

FIG. 3. Nearly adult *Syncalidium* viewed as a transparent object, showing the disposition of the nervous system.

FIG. 4. Adult *Syncalidium* viewed as a transparent object, but slightly diagrammatic, showing the gut, reproductive organs, and water-vascular system.

FIG. 5. Two egg-capsules of *Syncalidium*, showing their method of attachment to the gill-lamellæ, etc.

FIG. 6. Fragment of the ciliated membrane, showing its polygonal areolation; from a desiccated specimen.

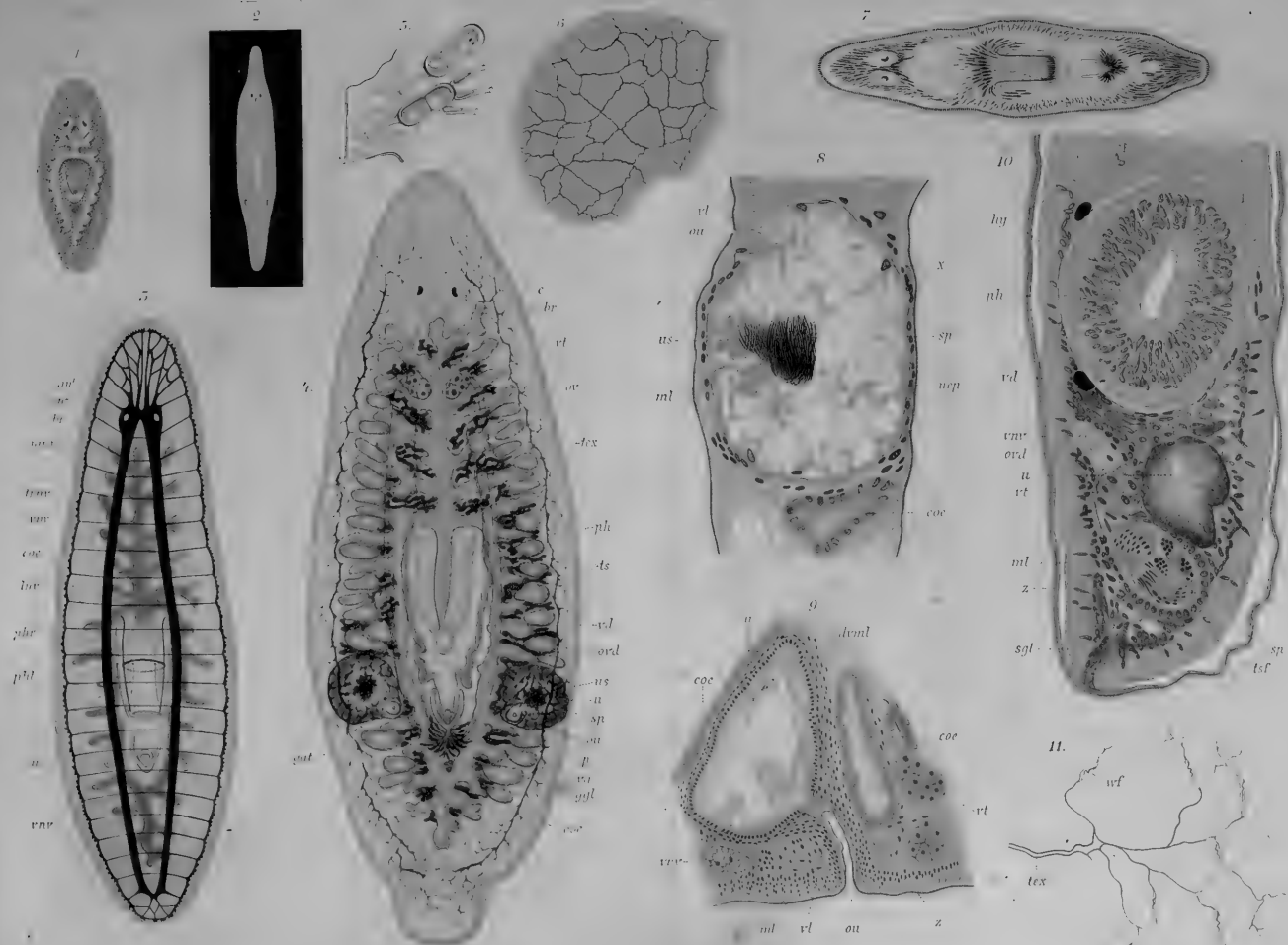
FIG. 7. Adult *Syncalidium* viewed as a transparent object, to show the distribution of the glands.

FIG. 8. Portion of a sagittal section through one of the uteri of *Syncalidium*, showing the duct and external opening.

FIG. 9. Portion of a transverse section through one of the uteri of *Bdelloura candida*, showing the duct and external opening.

FIG. 10. Transverse section of *Syncalidium* through the middle of the body.

FIG. 11. Fragment of the water-vascular system of a living *Syncalidium*.



PLANOCERA INQUILINA, A POLYCLAD INHABIT-
ING THE BRANCHIAL CHAMBER OF SYCO-
TYPUS CANALICULATUS, GILL.

WILLIAM MORTON WHEELER.

IN glancing over a recent paper by Verrill¹ on the marine planarians of New England—a work which the sea-side zoologist will find extremely useful—I find no mention of a curious Polyclad, which is nevertheless quite common in Vineyard Sound, Mass., the locality in which much of Verrill's material was collected. Having likewise failed to find any mention of this species in the writings of Lang² and v. Graff,³ I conclude that it is new. Perhaps the most interesting peculiarity of this new form, which undoubtedly belongs to the genus *Planocera*, as defined by Lang, is its parasitic mode of life. After reviewing all the cases of supposed parasitism among Polyclads, Lang concludes with the words (p. 630): "Kurz, es erscheint mehr als zweifelhaft, dass irgend eine der bis jetzt bekannten Polycladen wirklich eine parasitische Lebensweise führe." The new *Planocera*, however, seems to be a true ectoparasite, although I am not sure that it sucks the juices of the mollusk on which it lives.⁴ In all I have opened about 100 adult specimens of *Sycotypus*, and in the branchial chamber of nearly every individual from one to six of

¹ A. E. Verrill, Marine Planarians of New England. Trans. Conn. Acad. Vol. VIII Jan. 1893. pp. 79-140, Pl. XL-XLIV.

² A. Lang, Die Polycladen. Fauna u. Flora des Golfes v. Neapel. XI Monographie. Leipzig: Engelmann, 1884. pp. i-ix + 1-688. Taf. I-XXXIX.

³ L. v. Graff, Pelagische Polycladen. Zeitsch. f. wiss. Zool. 55. Bd. 2. Heft. pp. 189-219. Taf. VII-X. 1892.

⁴ Possibly, *Polochus zebra*, Verrill, may be somewhat parasitic. This beautiful species is not uncommon at Wood's Holl in the dead shells of *Lunatia heros* tenanted by hermit crabs and invested with *Hydractinia*. Whether the planarian merely selects the *Lunatia* shell as a hiding-place or bears some definite relation to the hermit crab, I am unable to decide. Verrill (*loc. cit.* p. 84) also mentions its occurrence in the "dead shells of *Fulgur* that were occupied by hermit crabs, *Eupagurus pollicaris*."

the Planocerae were found. They creep about slowly on the slimy walls and between the branchial lamellæ. To judge from the great accumulation of urates in their bodies, they probably feed on the nephric excretions, etc., so abundant in the branchial chamber.

My attention was first called to the Planoceran on *Sycotypus* by my friend, Prof. H. C. Bumpus, and I gladly acknowledge my indebtedness to him for helping me collect a number of specimens of this and other Polyclads while at the Wood's Holl Marine Biological Laboratory during the summer of 1892.

I append a brief description of the parasite together with some notes of a morphological nature.

Planocera inquilina, n. sp.

The firm and compact body is quite regularly oval. In the adult it measures 6 mm. in length by 4 mm. in breadth. Its edges are not raised and gracefully curled as in so many Polyclads, but remain in contact with the surface over which the animal is moving. Like many other planarians and pulmonate mollusks, *P. inquilina* is able to swim at the surface of the water in an inverted position. The ground color of the body is bluish or grayish and quite translucent, except in the center, where the pharynx (Fig. 1, *ph*) and reproductive organs (*u* and *sc*) are situated; these are opaque milk-white. Extending from this central mass very nearly to the periphery is a reticulum which is white in reflected and black in transmitted light (see right side of Fig. 1). In sections the substance possessing these color reactions is found embedded in the thick basilar membrane which immediately underlies the dorsal epidermis. I believe it must be some urate, although I have not applied the chemical test. Masses of the same substance are sometimes found enveloping the mature ova in the lumina of the uteri. It is the only substance in the body of the *Planocera* comparable to a pigment, excepting the true pigment in the eye-spots. In this absence of pigment *P. inquilina* resembles the Triclad's parasitic on *Limulus*. These latter have also lost the rhabdites, but in the mollusk parasite these

bodies are still present in the epidermal epithelium and in little nests in the parenchyma. The Biondi-Ehrlich stain proved to be very useful in making them conspicuous; the epidermal cells stain a faint rose color, whereas the rhabdites, like the

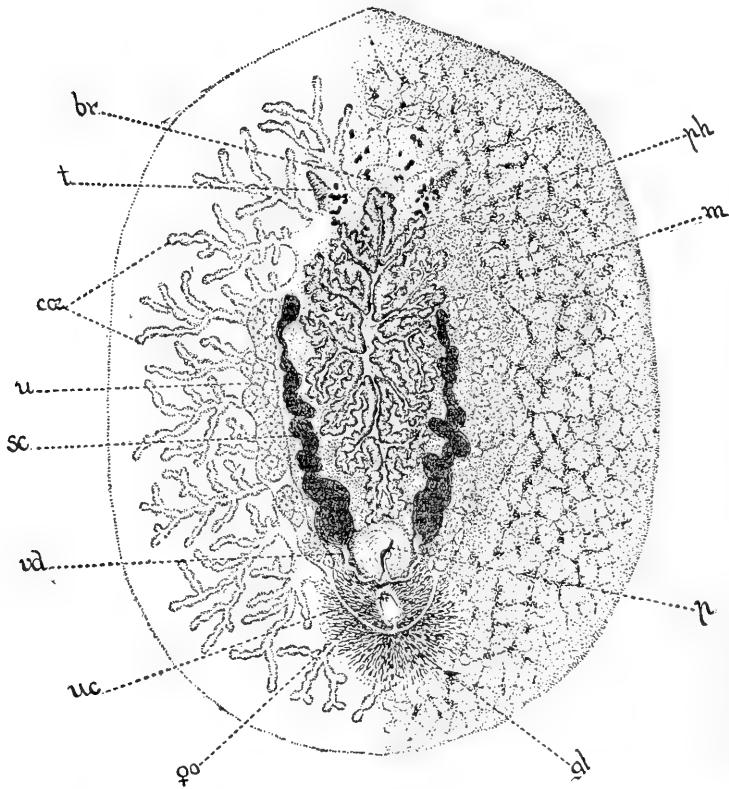


FIG. 1. *Planocera inquilina*, showing on one side the urate-reticulum; *m*, mouth; *ph*, pharynx; *coe*, gut-ramifications; *br*, brain; *t*, tentacle; *u*, uterus containing ova; *uc*, ducts of the uterus uniting to form the egg-duct; ♀ O, external opening of the egg-duct; *gl*, shell-gland; *sc*, seminal canal; *vd*, vas deferens; *p*, penis.

yolk-granules in the ova and the droplets in the cytoplasm of the intestinal epithelium, take on a deep orange color.

There are two exsertile and rather pointed tentacles (Fig. 1, *t*) on the dorsal surface about one-quarter the length of the body from the anterior edge. A collection of eye-spots marks

the location of each tentacle, part of these being embedded in the bases of the tentacles themselves. Two other more sporadic clusters of eye-spots lie in front of and nearer the median line, extending forward from the anterior edge of the brain. In most, if not all, other species of *Planocera* hitherto described, these mesial clusters extend much further back between the tentacles.

Owing to the lack of pigment, the nervous system may be traced without difficulty, especially in young specimens. It agrees so closely with Lang's description and figures of the nervous system of *Planocera Graffii* that I deem a consideration of it here unnecessary. It is perhaps well to note in passing that remarkably clear pictures of the beautiful plexus and its connection with the brain may be obtained by killing in hot corrosive sublimate, staining for 12 hours in Czokor's alum cochineal and, after dehydrating, mounting in gum sandarac dissolved in absolute alcohol.

The reproductive organs are interesting on account of their remarkable simplicity. I shall not describe the ovaries and testes and their collecting capillaries (Sammelcapillaren and Eileiter of Lang), as they appear to agree closely with their homologues in other Planocerids. The two large, convoluted spermatic canals (*sc*) run one on either side of the pharynx. They stain deeply, being puffed out with masses of mature spermatozoa in all the specimens which I have examined. Posteriorly they are continued as the vasa deferentia (*vd*) which unite in the median line near the hind edge of the penis-bulb. At their point of union they open by a very short duct into the styliform penis (*p*). I have failed to find any traces of a seminal vesicle or granule gland (Körnerdrüse), although I have devoted considerable study to sections and specimens mounted in toto. The absence of these two accessory male organs is noteworthy when their all but universal occurrence in the Polycladidea is borne in mind. According to Lang (p. 43) the granule gland is lacking only in the genus *Anonymus*.

The female reproductive organs are similarly simplified. The two uteri (*u*), which usually in the adult contain a considerable number of mature ova, are continued back as two delicate

canals (*uc*). These unite to form the muscular egg-duct (Eiergang of Lang). This opens (at ♀♂ Fig. 1) just behind the orifice of the penis, directly on the surface of the body, there being nothing which I can interpret as a bursa copulatrix. The numerous strands of the shell-gland (*gl*) converge from all points and open into the egg-duct.

In several of my sectioned specimens I was struck with the great number of spermatozoa in all parts of the body. Lang (p. 638) has also found them in many Pseudocerids, not only in the oviducts but also in the gut-diverticula and in the parenchyma. In *P. inquilina* the short typically Planoceran spermatozoa occur, not only in the tissues of the gut, pharynx, etc., but even in the substance of the brain and larger nerve-trunks. There is undoubtedly in this species a true "hypodermic impregnation," to use Professor Whitman's term.¹ In the aquarium the sexually mature animals crawl over one another and thrust their stylet-shaped penes into one another's bodies at any point. From this point, which may be found in sections, the spermatozoa travel through the tissues to the uteri. Spermatophores are not formed in *P. inquilina* as in leeches (*vide* Whitman *l. c.*) and some of the Polyclads (*e.g.* *Leptoplana*) described by Lang.

As soon as the mature ova pass into the uteri a curious phenomenon, first seen by Selenka² in the uterine eggs of *Thysanozoon Diesingii*, may be observed. The wall of the germinal vesicle fades away and a spindle is formed with distinct polar suns containing centrosomes.³ The small chromosomes, 9 or 10 in number, form an equatorial plate and appear to undergo fission, but of this I am not certain. Then the polar asters grow faint and vanish and the nucleus returns to the resting stage during or just before oviposition. Before

¹ C. O. Whitman, Spermatophores as a Means of Hypodermic Impregnation. Journ. of Morph., Vol. IV, No. 3, 1891. pp. 361-406. Pl. XIV.

² E. Selenka, Ueber eine eigentümliche Art der Kernmetamorphose. Biol. Centralbl. 1. Bd. No. 16. 30. Nov. 1881, pp. 492-497.

³ These last are pale and indistinct in *P. inquilina*, but in the Acœlan *Polychærus caudatus*, Mark, they are remarkably large and distinct. This occurrence of true centrosomes in the mature but unfertilized egg is of interest from its bearing on Boveri's well-known hypothesis.

the nucleus has returned to the resting stage the spermatozoon enters the egg. I have several times seen the deeply staining and somewhat twisted head of the spermatozoon lying in the cytoplasm near the arrested spindle. Further than this I have not traced the phenomena of impregnation, as my attention was first attracted to them while studying hardened material when I was far from the sea-shore. Why a spindle should be formed in the mature ovum and no division result, but only a return of the nucleus to its resting stage, is not easily understood. The spindle lies in the centre of the egg and has

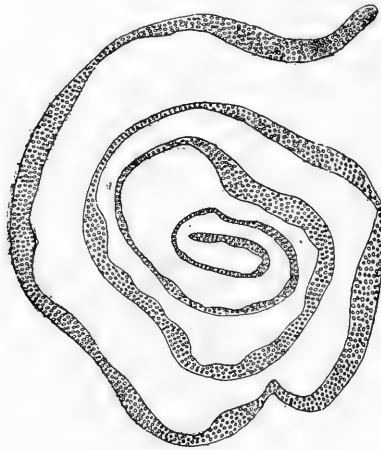


FIG. 2. Egg-string of *Planocera inquilina*.

nothing to do with the formation of the polar bodies; for these do not appear till some time after the eggs are laid, as I have several times had occasion to observe. Selenka implies that the aborting spindle has its *raison d'être* in bringing the scattered yolk particles to the center of the egg where they belong. In *P. inquilina* this certainly cannot be the object of the spindle, for the egg is full of yolk particles and there is no noticeable aggregation about the centrally located spindle. Lang, who has also observed the aborting spindle in the mature uterine eggs of all the Polyclads which came under his notice, has objected to Selenka's view on the very same ground.

Many specimens of *P. inquilina* deposited their ova in the glass dishes in which I kept them from July 28 to the middle

of August. The egg-string is spiral (Fig. 2), varying in breadth at different points and fixed quite firmly to the glass. It consists of a perfectly transparent gelatinous substance in which the small white eggs are embedded in a single layer. Each egg is inclosed in a delicate capsule probably secreted by the shell-gland surrounding the egg-duct. The helicoid shape of the string is evidently due to the animal's moving about in a spiral path during oviposition. Frequently the strings present more turns and more irregularities than the one figured.

I failed to follow the development of the eggs beyond the extrusion of the polar bodies and the first cleavage stages, as my material died. The water, though constantly renewed, was probably too warm, the mollusks, in which the eggs would normally have been laid, being found 6 or 8 fathoms below the surface. The animals themselves did not long survive in the aquarium. A slow process of dissolution set in at some point on the body, and gradually more and more of the tissue melted away till only the tentacle- and brain-region remained. This crept about for a few days, but finally it, too, disintegrated.

THE UNIVERSITY OF CHICAGO,
May 12, 1893.

THE ORIGIN OF THE SEX-CELLS IN HYDRACTINIA AND PODOCORYNE ; AND THE DEVELOPMENT OF HYDRACTINIA.

MARTHA BUNTING.

INTRODUCTION.

HYDRACTINIA was the name given by P. J. van Beneden to a hydroid which was found upon the coast at Ostend in 1841; so called because he considered it to resemble both the hydra and the actinia. It has since been described under various names, among which was that of *Alcyonium Echinatum* by Fleming; the latter specific name was accepted by Van Beneden, and thus, we have *Hydractinia Echinata*.

The form found by Louis Agassiz, in 1862, was called by him *Hydractinia Polyclina*, while Leidy also figured a similar hydroid, and named his after the European species. Agassiz founded his change of name upon very slight differences, among which may be mentioned a mouth in the reproductive hydroid, which he figures as frequently being opened very wide, and serving for the prehension of food. Also, that while found upon shells inhabited by *Pagurus*, it was obtained in great abundance in tidal pools attached to the rocks. Although I closely studied a large number of serial sections, the mouth was seldom demonstrated, but when seen, was extremely small. This small opening has also been observed, and figured both by Van Beneden and Weismann for the European species.

While found in great abundance upon *Pagurus* shell, I was unable to find them upon anything else; the shore would be covered by *Littorina*, upon which, however, no hydroids could be observed, but at the same place, when such shells were inhabited by *Pagurus*, they would in most cases be covered with these colonies; thus proving that the conditions in the latter were much more favorable for the development of the

Hydractinia than of the former. However, in the summer of 1891, Dr. Conklin tells me, that one of his students found them in great abundance upon some of the piles¹ of the Fish Commission wharf, but when searched for in the summer of 1892, there was no evidence of them remaining. Again, two students at the Wood's Holl Laboratory brought to me the shell of *Mytilus edulis*, and another of *Limulus*, upon which colonies were established. I carefully looked over the colonies and found that while the nutritive polyps were much extended and healthy looking, the reproductive ones were small and very immature, although at a time of year when the colonies on *Pagurus* shells were reproducing sexually with great activity. The colony, also, contained more of the defensive polyps in proportion to the other polyps than I had ever seen on *Pagurus* shells. The solution of this problem seems very clear, because when there was a choice offered, the planulae always showed a marked preference for *Pagurus* shells, while, if by chance, they should have drifted to a place where these shells could not be found, they were capable of developing upon anything else with which they came in contact.

The distribution of the colony upon the *Pagurus* shell is very interesting and suggestive. We find situated directly around the opening of the shell through which *Pagurus* protrudes, the long whip-like zooids, next to them the nourishing hydroids with a few reproductive ones interspersed among them, while beyond these, we have the reproductive polyps. This massing of the reproductive polyps gives a prevailing pinkish tint to the male, and a greenish tint to the female colonies. The position of the spiral zooids Weismann seems to consider a protection to the crab, in that they probably paralyze small animals and prevent them entering the shell. On the other hand, my observations lead me to believe that *Pagurus* is of immense service to the hydroid colony, and it is, perhaps, particularly due to this fact, that we find the hydroids so abundantly distributed upon the *Pagurus* shell. These

¹ Samuel F. Clarke found *Hydractinia* upon the piles of the wharf at Fort Wool, Chesapeake Bay. After close investigation he decided that this form was *Hydractinia Echinata*.

hydroids live upon animal food, and during the summer months when they are very prolific, it is not at all uncommon to find that these nourishing polyps had swallowed the young of *Pagurus* as they swam out from the shell; in fact, the whole colony, at times, assume a decidedly reddish hue, due to many of the hydroids having in their digestive cavity the undigested brilliantly colored *Paguri*. It can clearly be seen, then, that these hydroids obtain much of their food through their very convenient situation upon this *Pagurus* shell.

The study of the life history of the *Hydractinia* has been left almost untouched. P. J. van Beneden in 1841 observed the ova, which especially interested him, since he was able to find all the differentiation in them, which is to be seen in the ova of forms higher in the animal kingdom. Stethrill Wright in 1857, presented a short paper upon the development of *Hydractinia*, which brings out many salient points in its life history, although this paper is quite incomplete from our present standard. In this he states that the *Hydractinia* are "oviparous where true ova are discharged from the ovarian sacs, and subsequent changes into planaroid and polypoid forms take place after their leaving the zoöphyte." In his general description, he speaks of the ova carried in sporosacs that are developed from the hydrorhiza; he having failed to observe the blastostyle.

The development of the gonophore, and the origin of the sex-cells has been described by Ed. van Beneden in 1874, and also by Weismann in 1883.

PODOCORYNE.

A colony resembling the *Hydractinia* was found in the summer of '90 by H. W. Norris at Naushon, and brought by him for identification to Dr. J. Playfair MacMurrich, which by microscopical investigation I proved to be *Podocoryne Carneae*, first observed in 1839, and described in 1846 by Sars. In addition to the free swimming medusa, Sars speaks of hydroids bearing gemmae during March, but as I only had an opportunity to observe them in July, and August, I never found these organs.

Krohn, in 1851, describes this hydroid, and while he never finds any budding in the medusa, while attached to the reproductive hydroid, yet he finds adult medusae in the open sea, bearing a strong resemblance to the *Podocoryne*, and yet these bearing buds. The *Dysmorphosa Carnea* of Haeckel, seems to be similar to the *Podocoryne* obtained at Naushon and Wood's Holl, except the frequent occurrence of buds upon the four interrarial surfaces of the manubrium. This is a condition that I never observed, although having examined several hundred medusae; but these had come directly from the hydroid, instead of being found in the open sea. By skimming, very few were obtained, but those few had no buds, and resembled in all details those directly set free from the hydroid. The *Podocoryne* described by Grobben differs, in that, the medusa found at Naushon has but four marginal tentacles; the question arises, were the small interrarial ones overlooked by him, or was the medusa seen by him different from that described by Sars, Krohn and Haeckel?

The bell of the free swimming medusa found at Naushon and Wood's Holl, Pl. III, Fig. 21, was in height 0.5 mm. to 0.6 mm., the umbrellar margin from 0.2 mm. to 0.4 mm., the manubrium from 0.2 mm. to 0.3 mm. in length, although this latter varies, since it is so very extensible, at times, reaching even beyond the umbrellar margin. The tentacles are eight in number, four long perrarial, and four short interrarial. At the base of the tentacles are to be seen ocelli containing orange or reddish brown pigment, this is more marked at the perrarial than at the interrarial tentacles.

The shape of the bell varies, at times the aboral margin being much flattened, while at others it is rounded or convex. The umbrella is transparent and beset with numerous light points, which prove to be nematocysts. The manubrium contains a pigment resembling that of the ocelli; it is bounded by four mouth lobes, each containing bunches of nematocysts. At the four corners of the manubrium are situated the sexual organs. These are well developed, and appear quite mature, when the medusa is set free from the blastostyle.

The life history of *Podocoryne* was described in a preliminary

note by Varenne in 1882, in which he gave a short account of the development of the embryo and the origin of the sex-cells. In 1883, Weismann, in his work on the origin of the sex-cells in the Hydromedusae, gave a description of the origin of the sex-cells and development of this medusa, while in 1888, Ischikawa published a paper upon the female sex-cells of Podocoryne.

MATERIAL AND METHODS.

The material was obtained while studying at the Marine Laboratory of Wood's Holl, Mass., during the summers of '91 and '92. The shells, containing upon them both the Podocoryne and the Hydractinia, were found at low tide off Naushon and the Buzzards Bay side of the peninsula of Wood's Holl; while the Hydractinia was also obtained in great abundance at Wood's Holl upon the Vineyard Sound.

The surface views and segmentation were at first studied under the direction of Professors C. O. Whitman and J. Playfair MacMurrich during the summer of '91; to whom I extend my thanks for timely suggestions. The work was continued and completed under the supervision of Professor T. H. Morgan, for whose interest and willing assistance, I am very grateful.

The killing reagents, which have been found most successful out of the many experimented with, are "Kleinenberg's Picrosulphuric" in salt water, and Alcoholic Corrosive Sublimate. Both are equally good as to their preserving qualities, but the latter is much better, in that the staining process is more easily carried out after its use. The stains that brought out the cell structure in the clearest manner were Mayer's Picrocarmine and Kleinenberg's Haemotoxylin.

FORMATION OF THE GONOPHORE IN HYDRACTINIA, WITH THE ORIGIN OF THE SEX-CELLS.

The male and female sexual hydroids of Hydractinia, known as the blastostyles, arise from the hydrorhiza as special organs. It is possible to mark out the blastostyle wall into the regions mentioned by Van Beneden and Weismann as gastral *a*, germinal *b*, and cambium *c*, as represented in Pl. II,

Fig. 45, although there may be some exceptions to this rule in the position of the ova. The sex-cells reach maturity in gonophores, the larger and more advanced of which appear in the lower part of the germinal zone, while there is a gradual decrease in size, and age, as these pass towards the upper portion, shown for the blastostyle of both sexes, Pl. II, Fig. 47, female, Fig. 46, male.

The ova are found to be quite abundant in the endoderm of the blastostyle, even before there is apparently any trace of the gonophore. I have failed to observe in all cases that the older ova are found in the lower part of the germinal zone, the younger ones towards the upper part, as Weismann states. And I have found that they do not always rest upon the "Stützlamelle," thus "Man sieht hier, wie die Eizellen nach oben hin rasch an Grösse abnehmen, wie sie alle unmittelbar auf der Stützlamelle aufliegen, und wie die Bildung neuer Eizellen durch kleine Keimzellen vorbereitet wird, welche durch Theilung sich vermehren und alle ebenfalls auf der Stützlamelle fassen" (p. 77). I should conclude as a result of my studies that the ova, while frequently found in the position recorded by Weismann, are not unusually seen at the oral pole, even at the tentacles, as shown by Pl. I, Fig. 2; the younger ones in the lower part of the germinal zone, the older above as in Pl. I, Fig. 1; again I have not always found them upon the "Stützlamelle," as this figure also illustrates.

I have endeavored by close study to trace, if possible, these sex-cells to the ectoderm of the blastostyle, since Weismann assumes this to be most likely their true place of origin; but have failed to find the slightest evidence of their appearance in this layer, other than that the ectoderm in this portion of the blastostyle is closely crowded with nuclei, and becomes thicker in this region. Since the ova may appear at such different places in the endoderm, it seems rather safe to assume that the germ-plasm for the formation of the ova is present in the endoderm, and that while this germ-plasm has inherited a tendency to develop ova in a definite region, yet that under certain conditions ova may develop from it in other regions.

It is possible to look upon the multi-nucleated appearance of the ectoderm in the region of the germinal zone as an effort to prepare for the formation of the gonophore.

Both the female and male gonophores appear as outpushings of the ectoderm and endoderm (Pl. I, Figs. 3, 8, 9). Albert Lang, who, under Weismann, worked upon the budding and formation of the gonophores of the *Eudendrium racemosum*, *Eudendrium ramosum*, *Plumularia Echinulata*, and the *Hydra Grisea*, states, that the bud and gonophore come from the ectoderm alone, this layer forming both the ectoderm and endoderm of the new organ, the old layer of endoderm of the hydroid over this especial portion breaking down and disappearing. Since both Weismann and he suggest that this state of affairs may, in all probability, be found among all the coelenterates, I undertook to solve this problem for *Hydractinia* and *Podocoryne*. My results show that both layers take an active part in forming the gonophores; there are even karyokinetic figures frequently seen among the endoderm cells of the young stages, showing quite conclusively that this layer takes a part in this formation.

In Pl. I, Fig. 3, there is a breaking down of the supporting lamella preparatory to the formation of the "Glockenkern" from the ectoderm. This simple relation of the layers remains unchanged until they have pushed out much further. A later stage in the development of the gonophore we find represented in Fig. 4, Pl. I; here the ova are found still completely embedded in the endoderm, while a few cells from the ectoderm, *a*, *b*, have passed in between the two layers. Apparently the bell nucleus arises in this way from a few cells that later divide and form two layers. In Fig. 5, we see an adult condition of the gonophore, in which the ova have pushed out of the endoderm, but are still resting upon it. Thus, a small portion of each ovum is still bounded by the endoderm, while at least three fourths of each ovum is surrounded by the inner layer of the bell nucleus. The figures which have been given by E. van Beneden in his work on *Hydractinia*, represent the bell nucleus as consisting of two layers, but he considers that the ova still lie in the endoderm, and that the rounded ends alone protrude

from this layer and are bounded by the bell nucleus. Thus : "En fait, chaque œuf n'est un contact avec la lame testiculaire que cette partie agrandie de sa surface qui s'appliquait contre la lamelle hyaline, quand il était encore simple cellule ectodermique. Par toute le reste de leur surface les œufs voisins et les cellules de l'endoderm régénéré." It is to be noted that in Van Beneden's observations he looked upon *Hydractinia* as having been formerly hermaphroditic, and that the bell nucleus was in the female gonophore a degenerated sexual organ.

In Pl. I, Fig. 6, we have a distal portion of the gonophore shown, in which the external ectoderm layer forms a direct union with the endoderm of the spadix. I have never seen this appearance but once ; it may occur more frequently, however, as it is a rare chance to obtain an advanced gonophore in which so few ova are present, as the adult gonophores usually contain many ova, and thus such points could be easily hidden from view.

It was impossible to demonstrate the sperm mother cells in either the ectoderm, or endoderm of the blastostyle. Weismann speaks of observing groups of nuclei in the endoderm, which took a different degree of staining, and which he considered as the male sexual cells, but such a state of affairs I was never able to find. Pl. I, Figs. 8, 9, represent two sections of a series from the same gonophore ; the cells *a* and *b* are evidently just cut off from the ectoderm, and are to pass in to help form the bell nucleus ; they are more granular in appearance and stain more deeply than the other cells. Unfortunately I have never found karyokinetic figures in the ectoderm, which would place beyond a doubt the fact that the bell nucleus is formed by delamination from the ectoderm. The nuclei of the cell *d* (Fig. 9) appear to have just separated, but it is not evident enough to base any conclusions upon. I am led to believe, however, that the bell nucleus has an ectodermal origin, from the behavior of the cells *a* and *b* (Figs. 8, 9) towards stains, and from their position upon the supporting layer. Then, also, if we examine the ectoderm in the series of Figs. 8-13, all of which are drawn to the same scale, we shall see that this layer becomes much thinner, less granular, with

the small nuclei much further from each other, until finally it breaks down entirely, and the sexual cells escape. On the other hand, the endoderm remains quite as thick, the nuclei become smaller, but they are correspondingly more numerous. The fact which remains to be decided is whether the bell nucleus originates as a whole by delamination, or whether the cells migrate from the ectoderm and divide afterwards.

The cells that pass in from the ectoderm, very soon arrange themselves in a double layer. (Fig. 10, *c* and *d*.) We here see the bell nucleus consisting of the two layers closely applied to one another. There seems to be no stage where we find either an infolding of the ectoderm, or a solid mass of cells, which in later stages form into these two layers. In Fig. 11, we see that the double layer of Fig. 10, has become differentiated into an outer one consisting of a single row of cells, and an inner one made up of several rows of cells which stain much more deeply, and are more granular; these are evidently the spermatoblasts. In the series Figs. 11, 12, 13, we see the direct formation of the spermatozoa. In the first figure the spermatoblasts are undergoing division, which is shown by the spindle. Karyokinetic figures are of frequent occurrence in sections of gonophores at this period of development. This fact teaches that by karyokinetic division of these cells the later stages are reached. As the series advances, an increase in the number of cells and a decrease in the size is noted, until finally, we reach, in Fig. 13, a stage where the minute chromatin heads with delicate protoplasmic tails are found; these are the mature spermatozoa. Van Beneden in his Pl. II, Figs. 12, 13, 14, shows that this spermatogenesis takes place within the inner layer of the bell nucleus. He, however, considers that this layer is not entirely used up by the formation of these sex-cells, but remains as a very thin layer containing very minute nuclei, and thus, after the mature spermatozoa are developed, the double layer of the bell nucleus may still be observed. I cannot deny, or affirm, that such a layer was present, for although, at times, I thought I could detect it, yet more frequently there was no evidence whatever of its existence.

Van Beneden has shown in *Hydractinia* that the external ectoderm folds in to form the bell nucleus. I, therefore, studied my sections with great care; after which several hundred blastostyles were examined from surface views, made possible by means of clearing with clove oil. The lesson which this investigation seemed repeatedly to teach was, that a few cells were cut off from the ectoderm at a certain position, and these cells gradually increased to form the double-layered bell nucleus in which a cavity never existed, the layers always remaining closely applied to one another.

FORMATION OF THE BELL NUCLEUS OF *PODOCORYNE*, AND ORIGIN OF SEX-CELLS.

The blastostyle of *Podocoryne* is, as has been mentioned, much smaller than the nourishing polyps, although still bearing a marked resemblance to them. According to Weismann a germinal zone does not exist, but rather a "Knospungszone," which is found at about the same area. The origin of the ova from the endoderm of the young blastostyle before the appearance of the medusa buds, was not seen, as was observed by Varenne. I finally came to the conclusion of Weismann, that possibly Varenne had mistaken for ova the darkly staining cells which are frequently met with in the endoderm; these are without doubt glands, as they are also present in the nourishing polyp.

The medusa-bud in *Podocoryne* appears first as an out-pushing of both ectoderm and endoderm. In the female bud represented in Pl. I, Fig. 14, we observe ova of different ages lying in the endoderm. As the bud progresses in its development, we find the ova in the endoderm of the manubrium. When a later stage has been reached, they migrate from the endoderm to the ectoderm, as is clearly shown in Pl. I, Fig. 16, in which *a*, *b* are in process of passing through, while *c*, *d* are in the ectoderm, and *e* still in the endoderm. A small drawing of the entire bud is given in Fig. 17, to show the orientation of the manubrium. In the adult state the ova are always to be found in the ectoderm. In one case an ovum was

found situated at the base of the medusa-bud (Fig. 18). Ischikawa speaks of occasionally seeing what he considers ova in the ectoderm, and states that he thinks this an additional reason for the supposition held by Weismann, that the sexual cells do, in fact, come from this layer. It certainly seems to point very strongly in this direction, as we could imagine that some germ-plasm had failed to pass through the supporting lamella into the endoderm, and thus had proceeded with its development while in the ectoderm. On the other hand, it may simply mean that germ-plasm is present in the ectoderm, as well as in the endoderm.

The male medusa-bud is formed similarly to that of the female; and in Fig. 19, we see an early stage in the development of the bud. It is noticeable that in the ectoderm, there are numerous nuclei at the apex, a condition of affairs which is frequently met with, and has, I find, been noted by Weismann. This leads me to believe that the multiplication of the nuclei is taking place prior to the formation of the bell nucleus.

The spermatozoa originate in the ectoderm of the manubrium, arranged, as are the ova, in four masses (Pl. I, Fig. 20). A small drawing showing the orientation of this manubrium is given in Fig. 21.

It was my desire to follow out the cleavage and further development of the egg of *Podocoryne*, but it seemed impossible to keep the medusa alive long enough to obtain their eggs; and then again, the colonies were so few in number, that usually but one single colony was found among fifty of *Hydractinia*, and on account of this scarcity, only a male or female colony would be obtained at one time.

In the formation of the medusa the bell nucleus appears as a solid mass which pushes the endoderm before it (Fig. 15). There is, later, a separation in this ectodermal mass of cells, forming an external layer, and an internal mass of cells; this mass, also, afterwards, separates into two layers, a middle and an inner layer, with a cavity between them. As development progresses, the external layer of ectoderm becomes more and more attenuated at the oral pole, until finally, both it and the middle ectodermal layer break through at this point. The

terminal cells of these two layers then unite, and we have an appearance as though the umbrella had been formed by the infolding of the external ectoderm. At about this same time the velum forms, and the medusa with all its organs complete, and containing the adult sexual products, is now ready to escape from the blastostyle, and lead an independent existence.

THEORETICAL.

The discovery that, at Wood's Holl, colonies of *Podocoryne* and of *Hydractinia* are found side by side under similar conditions of life, and yet appearing in such different proportions, is of much interest. Surely, there is opportunity to learn here a lesson in the phylogenetic history of the *Hydromedusae*. What are we to conclude? Is *Hydractinia* very slowly developing into the more complicated form of *Podocoryne* with its free swimming medusa, or is *Podocoryne* gradually forming the hydroid with the retrograde blastostyle and gonophore? We must consider which is best adapted to survive in the struggle for existence. The sex-cells carried in these very small medusa subjected to the influence of the waves, would be much more likely to fall into places unfavorable for development than would the sex-cells carried in the gonophore. Moreover, we have evident signs of degeneration in both blastostyle and gonophore of *Hydractinia*, to quote Dr. W. K. Brooks: "In *Hydractinia*, the cormi of which are so similar to those of *Podocoryne* that a drawing of one will correctly represent the other, the life history begins to simplify itself by the degradation of the sexual medusa into sessile buds, or reproductive organs which, however, still retain traces of their former independent locomotor existence."

Sidney Hickson takes a different view of the subject; while not speaking directly of *Hydractinia* and *Podocoryne*, he traces the gradual development of the medusa through the gonophores of forms similar to *Hydractinia*, from the simplified gonophores of *Allopora* and *Distichopora*.

Until more evidence is collected to support this view, it seems more logical to adhere to that which considers *Hydractinia*

as degenerate, and that the gonophore represents a retrograded umbrella, rather than a step in the phylogenetic history of the more perfect medusa.

Weismann considers the "Keimstätte" as existing in four different positions, due to a shifting process: first, the sex-cells originating from the ectoderm of the manubrium; second, from the ectoderm of the bell nucleus; third, from the endoderm of the manubrium; and fourth, from the endoderm of the blastostyle. Assuming that the first state is the primitive condition, we have the ova of *Podocoryne* belonging to the third, those of *Hydractinia* to the fourth. While the spermatozoa of *Podocoryne* are still found in the first state, those of *Hydractinia* according to Weismann are found in the fourth, while as a result of my studies, I place them in the second state. Thus Weismann looks upon this shifting of the germinal states as a proof of the origin of *Hydractinia* from *Podocoryne*.

That the sex-cells when mature are found in the ectoderm of the manubrium and spadix in *Podocoryne* and *Hydractinia* respectively, proves, Weismann thinks, that this was phylogenetically their origin and position of complete development, thus for *Hydractinia* he says: "So kann z. B. bei der weiblichen *Hydractinia* diese Wanderung ins Ektoderm des Manubriums schwerlich noch einen andern Sinn haben, als den einer phyletischen Reminiscenz, einer ontogenetischen Wiederholung der Stammesgeschichte."

THE UNSEGMENTED EGG.

In *Hydractinia* it was found that the ova and spermatozoa were discharged about the same time, namely from 9.30 to 10.30 P.M. The ovum while in the gonophore has a well defined nucleus situated above the center of the egg, which fades from view when the ovum is deposited. The mature eggs when observed by reflected light have a greenish tint, and are not surrounded by a vitelline membrane. Sections of the egg show deutoplasm spheres distributed throughout the protoplasm, with the exception of an outer rim which is composed entirely of protoplasm.

When first laid, the egg is sometimes oval, although usually globular, having an average diameter of 0.16 mm. On being discharged, the ova sink at once to the bottom of the vessel in which they are contained. Experiments seem to show that the ova are fertilized either just previous to, or at the moment of their ejection. In about fifteen minutes after the ova are laid, the polar bodies (Pl. II, Fig. 22) appear. When first observed two globules were present, one had been extruded, while the other one was just appearing. One of the two divided subsequently, in a plane at right angles to the first cleavage plane of the ovum (Pl. II, Fig. 23). Within ten minutes from the extrusion of the first polar body, the second was ejected.

CLEAVAGE.

The deutoplasm and protoplasm being evenly distributed in this ovum, there is no marked division into an animal and vegetative pole.

The first cleavage takes place about forty minutes after the egg is laid. The plane of division is vertical, cutting in from the point where the polar bodies pass off, and dividing the egg into equal right and left halves. The first furrow, when viewed from above, appears as a cup-shaped depression, as is seen in Pl. II, Fig. 22. This progresses slowly, until at the end of ten minutes, the two blastomeres are simply connected by a slight protoplasmic thread (Pl. II, Fig. 24). In this thread protoplasmic movements are often seen. At the end of fifteen minutes, the complete two-celled stage is reached.

The second cleavage planes start from the center about a half an hour after the first cleavage begins, and work their way gradually to the outside of the egg. During this cleavage there is frequently a movement of the blastomeres, which completely changes their relative positions to each other. At the completion of this cleavage they again assume very nearly their former positions. At first they appear as four cells around a central cavity as is shown by Dr. E. B. Wilson in Diagram VIII, *A*, of his paper upon *Nereis*, for the four-celled stage of the true radial type, represented by *Amphioxus* and *Synapta*;

excepting that these two sets of blastomeres do not lie directly opposite each other, but have undergone a slight rotation. These cells flatten down and fit together at the center, the cell a^1 of the primary blastomere *A* coming directly in contact with the cell b^1 of the primary blastomere *B* (Pl. II, Fig. 26). When viewed from the opposite side we would find a reversed condition, the primary blastomeres *A* and *B* forming the connection with one another. We here see the "cross furrow," which is so often found in eggs at this period of segmentation. In one half hour after this cleavage begins, the complete four-celled stage is reached.

The third cleavage commences about one hour and a quarter after the appearance of the first cleavage. Each blastomere divides separately, the cleavage, as before, starting in the center and running centrifugally. There is evidently a decided rotation to the left at this stage. In fifteen minutes from the beginning of this cleavage we have eight cells formed; there are, however, as yet five in one group, and three in the other; gradually one sinks down, and we have the complete eight-celled stage, in which there are two groups of four cells lying directly across one another. While the cells of this stage usually assume this position, there is considerable variation found at this period.

As before, without a period of rest, the fourth cleavage appears and runs centrifugally to form the sixteen-celled stage. This stage is reached about an hour and forty minutes from the appearance of the first cleavage plane. When viewed from the pole shown by Pl. II, Fig. 29, we find that the four cells occupying the most prominent position are derivatives of cell *B*, Fig. 24.

The cells become so small, and are so opaque, that it is impossible to follow the cleavage further. This also seems unnecessary, since it appears to be total and equal; and thus impossible to trace the formation of organs to any especial cell.

In those forms which Metschnikoff has studied, he notes that the centripetal and centrifugal methods of cleavage remain constant for the different species, and that in this way the different eggs could be distinguished, even if contained in the

same vessel. He has also observed that this method of beginning at one pole and passing through to the opposite one, ("Schneidende"), is to be found among the metagenetic medusae; while the circular method is to be observed in the hypogenetic forms.

In about two and one half hours a spherical mass of cells (Pl. II, Fig. 30) is formed, which is motionless. In ten or eleven hours after the first cleavage this elongates, becomes at first oval in shape (Fig. 31), still opaque, and having, when viewed by the reflected light, the same greenish tint as the ovum. The embryo remains unciliated and motionless, for about sixteen or seventeen hours, when we find the opaque planula gradually revolving; we see later that it becomes further elongated (Fig. 32) and pinkish in tint, resembling the color of the adult animal. When viewed by transmitted light, we find the embryo to have an inner mass surrounded by a slightly transparent zone, in which numerous nematocysts appear; these are collected in greater numbers at the tapering end where the cilia are shorter, while the forward directed blunt portion contains longer cilia and fewer nematocysts. In about thirty-six hours, the ectoderm of the broad end has much increased in thickness, preparatory to the formation of a disc to serve as a means of attachment. The planula becomes so attenuated and tapering, that it very much resembles a tentacle of the adult hydroid.

After thirty-six to forty-eight hours, those planulae that were to develop further became attached at the broad end; they then contracted, remaining in this position for varying periods of time; under most favorable conditions they became hydroids in about four days. From the base of the hydroids we find at first "stolon-like" outgrowths (Pl. III, Fig. 64) that later are to form the hydrorhiza. The tentacles first appear as projections from the wall of the hydroid. The mouth has been formed in the hydroid with four tentacles, as is shown by a surface view in Fig. 65, and the hydrorhiza now consists of four tubular outgrowths (*h*). In a six-tentacled polyp we see these tubes increased in length, with one turned up as though about to form another polyp (Fig. 66).

I have carefully observed about fifty young hydroids of *Hydractinia*, all of which were nutritive polyps. I, therefore, infer that this polyp is always developed before the protective and reproductive forms. In one instance, I saw a hydroid about nine days old with its hydrorhiza, from which a reproductive polyp (Fig. 67, R), with its knob-like tentacles, had developed. Unfortunately, it was mutilated in being removed from the dish, to which it closely adhered. Therefore it became impossible to examine it thoroughly, and thus to give absolute proof of the fact that the reproductive forms are developed in this way, and at such an early period.

ENDODERM FORMATION.

Since the form of cleavage is centrifugal to such a marked degree, there is a tendency in the very early stages of segmentation for the cells to arrange themselves as a layer around a central cavity; which has also been noted by Metschnikoff for *Clytia*, *Liriope* and *Nausethoë* (p. 41). In *Hydractinia*, when the embryo consists of about sixteen cells, the blastula is formed. By a careful study of a number of serial sections karyokinetic figures are observed at this time, which seem to indicate a delamination to form the endoderm (Pl. III, Fig. 48). In Figs. 49, 50, are shown two sections of a series in which the spindles are seen in different cells. These show that the endoderm formation takes place by multipolar delamination, even at this early period in the development. The inner portion of these cells are cut off, and pass into the blastula cavity. During this period, by karyokinetic division, the cells of the blastula wall are also increased in number (Fig. 51). Karyokinetic figures are still to be observed in later stages in cells situated at different points of the blastula (Figs. 52, 54), showing that multipolar delamination is continued for some time, until in cross section, the embryo has its outside wall consisting of about twenty cells.

In some sections we see that a cell will appear as though about to migrate into the cleavage cavity (Fig. 54, *b*). If this took place we would have, according to Metschnikoff, a mixed

delamination. This method of origin of the endoderm has lately been noted by Brauer for *Hydra*, where he finds multipolar delamination, mixed with an inwandering of cells. By examining the next section in the series, however, the spindle is always found, which demonstrates that there is simply an elongation prior to delamination (Fig. 55, *b*). The endodermal cells divide karyokinetically, as well as the blastula cells, until we have a solid mass of cells formed. This consists of two layers, an external one-celled layer and an internal mass of cells (Fig. 56).

FURTHER DEVELOPMENT OF THE EMBRYO.

At about twenty-three hours, we find the endoderm beginning to hollow out (Fig. 57), although it is still several cells in depth. The ectoderm is composed of a single row of columnar cells intermingled with numerous gland cells. There is a very sharp outline formed between these two layers, which gradually increases, forming the so-called "supporting lamella." Longitudinal sections show that this cavity, which has arisen by the breaking down of the endodermal cells, has, as yet, no connection with the exterior. Also we find that the embryo has elongated. The digestive cavity remains closed to the exterior for several days. Fig. 58 shows a section of a planula which has been attached at point *A*, for four and one half days; the coelenteric cavity *C*, is still closed.

The mouth pole assumes the conical shape of the hypostome, while the tentacles appear as outpushings of both ectoderm and endoderm (Fig. 59). A step still farther in the development is shown in Figs. 60, 61, both of the same hydroid in which the coelenteric cavity has opened to the exterior. The tentacles are solid, consisting of a layer of ectoderm surrounding a single row of endoderm cells. The cavity of the hydrorhiza (*D*) is found to be in connection with the main digestive cavity. The hydrorhiza forms the coenosarcal tubes which connect the different members of the colony; and later, a secretion from this coenosarcal base forms the chitinous ground work, with its many protruding spines.

ABNORMALITIES.

In the cleavage of the egg many peculiar forms of segmentation appeared, but it was impossible to demonstrate that they all formed planulae. Those that were followed did not; yet as very frequently even those having normal cleavage died while under observation, we cannot conclude that under no circumstances do these abnormal forms become hydroids. The most common abnormality is that arising at the first cleavage, in which the plane does not pass entirely through the egg, but buds off two, three or four small cells at the upper pole, leaving one large cell at the lower pole. Dr. E. B. Wilson mentions this form of cleavage in *Renilla*. Thus in enumerating the forms of cleavage found in this animal, he states "cleavage may begin at one pole of the egg with the formation of four or five small spheres, and usually after a quiescent period the remainder of the vitellus breaks up at once or progressively into spheres approximately the same size as those first formed, and the egg passes into the sixteen-sphere stage." Again Brauer also notes for the *Tubularia Mesembryanthemum* Allm.: "Im allgemeinen verläuft sie auf zwei verschiedene Weisen, entweder folgt jeder Kerntheilung auch eine Zelltheilung, oder es vermehren sich zunächst nur die Kerne und es beginnt dann eine allmähliche Abfurchung, welche am Richtungskörperpole anfängt und dann nach der entgegengesetzten Seite fortschreitet." I have once thought I detected the large cell breaking up into small cells, but at other times I have watched this form for three and four hours without any change taking place in it.

In starting to form the four-celled stage the blastomeres frequently undergo a movement, at times taking a position in which instead of lying opposite one another they lie beside each other, their furrows apparently cutting in from above as in the first cleavage (Pl. II, Fig. 33). In this case by further segmentation a chain of loosely detached cells is formed (Figs. 34, 35). I did not, however, trace the development beyond this stage. Metschnikoff has found this plan of

segmentation in the *Oceania armata* Köll., in which he traces it to the planula (Pl. I, Figs. 34, 35).

That in some instances these irregularities in segmentation might affect the planular stage, was suggested by the fact that very many peculiar forms of planulae were found intermingled with the normal ones. The most noticeable among these was one having an anterior blunt end and two posterior pointed ends giving it a λ shaped appearance (Fig. 36); others consisted each of two processes united simply at their anterior ends, their two processes extending directly out from one another like two wings; again the anterior end of one planula would be attached to what appears to be the posterior end of another one; others would appear with a constriction in the center, giving the planula a somewhat dumb-bell shape; often these latter ones would be observed in pairs lying directly across one another. In all these forms each portion was about half the normal size, strongly pointing toward the fact that they were derived from the same ovum. Those planulae having a blunt anterior end, with two pointed posterior ones, attached themselves to the dish at the broad end. I watched them in order to find out, if possible, whether divisions into individuals appeared thus early in *Hydractinia*, or whether twin hydroids would be developed. Although carefully observed for a week, and placed under favorable conditions, the development proceeded no further; but this is not to be regarded as indicating that development was impossible, since an enormous percentage of the normal planulae did not develop beyond the attached planular stage.

ADULT ABNORMALITIES.

The reproductive polyp with adult gonophores was found in one instance as a bud coming off from the nutritive hydroid. Hincks has figured a bifurcating zooid of *Hydractinia*, in which there is a nourishing polyp and a reproductive one, although the latter has no gonophores; while Agassiz has shown a bifurcation of the spiral zooids.

In a few cases fission of the nutritive hydroid was also seen; thus besides the usual sexual method of development, we find

budding of the sexual polyp from the nutritive polyp, and longitudinal fission playing a somewhat inconspicuous part.

In a section cutting two male gonophores upon the same blastostyle, there was found in two cases ova among the spermatozoa. On one side of the spadix would be an ovum, while upon the opposite side, the spermatozoa would appear (Pl. I, Fig. 7). A great number of serial sections, about one hundred, had been carefully studied previously without meeting this state of affairs.

In *Podocoryne* I found a twin medusa; this condition has also been observed by Grobben. Each medusa seemed to have the normal number of tentacles, and to be about the normal size. They were attached along the margin between the radial canals.

Amongst the nutritive polyps of *Podocoryne*, two of normal size were found apparently in the act of longitudinal division, or of budding; also a nutritive polyp, with a reproductive one developing upon either side; thus it seemed possible that fission or budding might take place both in the hydroid and in the medusa.

EXPERIMENTAL.

Under normal conditions the ova were discharged from 9.30 to 10.30 P.M.; and this necessitated the study of the early segmentation stages at night. I tried to make it possible by changing the conditions to change the time of deposit of the eggs. The *Paguri* were removed from *Littorina* shells, leaving these with *Hydractinia* upon them; they were then placed in a dish of salt water which was carefully packed in ice at 8 P.M., August 17, 1892. A registering thermometer was placed within the refrigerator and found not to have recorded a lower temperature than 17.5° C. The dish was removed at 8 A.M., August 18. The temperature was 24° at 9.20 A.M.; at this time the discharge of ova and spermatozoa from the colonies took place; segmentation began in about an hour.

On August 18, the same colonies that had been exposed to the ice on August 17, were exposed to the direct heat from a lamp at 8.30 P.M., and ova were laid at 9.15 P.M. These did

not segment, which was probably due to the unusual heat to which they were subjected.

Colonies that had been observed under normal conditions on August 17, were placed upon the ice the night of August 18, at 8 P.M. Removed from the ice at 9.30 A.M. on August 19, when the eggs and spermatozoa were discharged; these eggs segmented normally at 11.10 A.M.

The experiment of lowering the temperature was tried again on August 19. The shells with the colonies upon them were removed in three series on August 20; one at 8 A.M., the second at 10.30 A.M., while the third at 2.10 P.M. In the first series, eggs were laid, and segmentation started in about an hour to an hour and a half after removal from the ice, while in the second and third, immediately upon removal from the ice the eggs were deposited and segmentation commenced.

On August 20, the experiment was again repeated, the removal from the ice being done at different periods of time. The outcome of these experiments made it possible to watch the segmentation by daylight, and the material could be studied at different times. This was of importance, since one of the serious difficulties had been that the cleavage began simultaneously in almost all of the eggs, those which had been belated proving unsatisfactory for observation. This method of placing the entire colony upon ice some time before the period at which the eggs were to be discharged, was suggested by Mr. Bristol, who had tried it with some success on other forms.

Through various experiments in the summer of 1891, the time for the normal deposition of the eggs seemed to be at about 10 o'clock P.M.; therefore, at that time they had been carefully examined by lamp-light, and it was found that about 11 o'clock was the time at which normal segmentation took place. The fact that the colonies had been subjected to strong light, after having been kept in the dark, both in this case and in that when they were placed upon ice, led me to question whether the discharge of the ova and spermatozoa could be due to the effects of the light. Therefore, immediately upon removal from the ice some colonies were placed in complete darkness. Upon observation, later, it was discovered that this

had not retarded their action, as their action was the same as with the others, which had been removed from the ice at the same time, and exposed to the light.

My experiments lead me to formulate the opinion that the ova and spermatozoa are ripened and ready for expulsion about 10 o'clock P.M. ; and that by chilling the whole colony for a sufficient length of time before this period, it is possible to delay the time of deposit.

Experiments were tried in order to learn just when the ova were fertilized. The male and female colonies placed upon ice were kept separate over night. Artificial fertilization was then tried by adding some water from the male colonies containing spermatozoa to the ova of the female colonies which had been deposited, but no segmentation followed excepting in a few cases, where there was a beginning of the first furrow. In another experiment, the male and female colonies were separated immediately upon removal from the ice, and the water changed before there was evidence of the ova being deposited, yet they segmented. Therefore it is to be supposed that the spermatozoa either fertilize the ova just as they are about to escape, or at once upon their discharge, probably before they have sunk to the bottom of the dish ; yet it is possible by microscopical examination to see an ovum surrounded by spermatozoa, after it has so settled.

At Wood's Holl on June 25, the *Hydractinia* which were first collected, showed a much larger percentage of male colonies than of female ones. During the latter part of July, and August, when the temperature of the water had risen, and the conditions for nourishment were more favorable, there was an excess of female colonies. There was not sufficient data obtained to prove conclusively that temperature and nutrition influenced the determination of sexes, but the difference in their proportion with regard to these conditions suggested that temperature or nutrition were the causes for this differentiation.

The work of Driesch upon *Echinus*, and Roux upon *Rana*, suggested the following experiments upon *Hydractinia*. In several instances at the two-celled stages, the blastomeres were

shaken apart. Often the two separated blastomeres were found to be irregular in shape, but they each became spherical in a short time. In the blastomeres separated at the two-celled stage, the cleavage progressed as in the normal process, and with the same intervals, up to the eight-celled stage. (Figs. 37-42.)

There were comparatively few of those shaken, or cut apart, which developed to the planular stage; but as the number was relatively small upon which the experiment was tried, and as there is a large percentage of death amongst the ova and embryos, even under normal conditions, it cannot be asserted with certainty that the separation has such a deleterious effect as to greatly increase the death rate. The planular stage is somewhat delayed, also the planulae much smaller in size than the normal embryo. In Fig. 43, we have an elongated embryo of about eighteen hours, which is still opaque and unciliated, while the normal embryo is swimming at this time. In three days, we have a planula swimming very slowly, and rather peculiar in shape (Fig. 44). This had become pinkish in tint in about twenty-six hours, and it is possible that it may have moved sooner, although so sluggishly, that I had failed to observe the movement before this time.

Although I have searched among the few sections I possess of these half embryos, to determine whether the endoderm arises through multipolar delamination, as in the normal embryo, I have been unfortunate in not being able to satisfactorily demonstrate this. That the endoderm is formed by delamination is shown by Pl. III, Fig. 62, in which a karyokinetic figure is seen; this might indicate unipolar delamination, but we have every reason to believe that the origin of the endoderm follows the same general plan as in the normal embryo. Fig. 62 shows that the ectoderm cells then form into a row of columnar cells, with a sharply defined outline between them and the endoderm cells, and that the latter break down to form the central cavity.

The four blastomeres were separated at the four-celled stage. These quarter embryos later became four-celled, but they were so small that I was unable to follow the segmenta-

tion beyond this point. There were no quarter planulae obtained.

In order to find out whether when the two blastomeres of one ovum were separated they divided synchronously, a very fine needle was carefully sharpened until a small knife edge had been formed, and then the two blastomeres cut apart with this. At first they were flattened each on one side where they had been in contact, as is shown in Pl. II, Fig. 37; in five minutes they became globular (Fig. 38), and in ten minutes for both blastomeres the first cleavage (or properly the second cleavage) started in one, forming the two-celled stage, while in the other it did not pass through to the opposite pole, but started to form buds. Again, in ten minutes more, the second cleavage appeared, and while in one blastomere this resulted in the formation of a four-celled stage perfectly normal in appearance; in the other one a constriction formed that cut off buds, making three small cells at the upper pole, and one large cell at the lower pole; both of these conditions were completed at the same period of time. As this latter form is one often seen under normal conditions, we cannot conclude that this was due to any injury caused by the separation.

Another fact which was very noticeable in these blastomeres which had been shaken, or cut apart, was, that the blastomeres derived from them, seemed to have lost, at times, their power of holding together. Very often they would segment for a certain distance, and then, the cell mass would break up into numerous minute blastomeres.

I regret that I was not able to separate more of these eggs, and to follow this work further, but it was not until the last week of my stay at Wood's Holl that it was found possible to observe them other than at night, and as the segmentation was carried on so rapidly, there was not as much opportunity for repeating the experiment as often as desired. However, since in five cases planulae were reached, it seems to prove that in *Hydractinia*, as Driesch has proved for *Echinus*, and E. B. Wilson has shown for *Amphioxus*, each blastomere of the two-celled stage may develop into a half embryo.

CONCLUSIONS.

The gonophore of *Hydractinia* arises through the protrusion of the ectoderm and endoderm caused by a multiplication of cells in both layers. The bell nucleus does not originate by invagination, but from a few ectodermal cells, that take up a position between the ectoderm and endoderm, and increase, forming subsequently two layers of cells.

The ova are first observed in the endoderm of the blastostyle, thus, apparently endodermal in origin; reach maturity on the outside wall of the spadix, lying between the endoderm and the inner layer of the bell nucleus. The spermatozoa arise from the inner layer of the bell nucleus; we see that they are, therefore, ectodermal in origin.

The medusa-bud of *Podocoryne* arises as a protrusion of the ectoderm and endoderm, caused by an increase in the cells of both layers. The bell nucleus is formed from ectoderm, not by invagination, but consists at first of a solid plug of cells; later, by separation of these cells there is formed two layers with an intervening space.

The ova are first seen in the endoderm of the manubrium, and they reach maturity in the ectoderm of the manubrium. The spermatozoa arise in the ectoderm of the manubrium, and reach maturity in the same position, thus, they are ectodermal in origin.

The ovum of *Hydractinia* is laid at a definite period of the day; two polar globules are formed; both leave the surface of the egg at the beginning of segmentation. Segmentation is total and equal. The endoderm originates through multipolar delamination, beginning at about the sixteen-celled stage, and *cells continue to delaminate for some time*. By breaking down of endoderm cells, the coelenteric cavity arises, at the same time the two layers are sharply differentiated and the supporting lamella appears. The embryo becomes elongated and ciliated; attached to a substratum by its blunt anterior end. The tentacles appear as solid endodermic outgrowths surrounded by ectoderm. The mouth is formed by the coelen-

teric cavity breaking through to the exterior. The hydrorhiza appear first as stolon-like outgrowths, later they become tubular.

By lowering the temperature, the period of time for the egg laying and ejection of the spermatozoa may be changed.

Blastomeres separated at the two-celled stage develop half planulae.

BRYN MAWR COLLEGE, April 22, 1893.

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EXPLANATION OF PLATE I.

[Figs. 1-20 (with exception of 7 and 17) $\times 372$; 7 $\times 187$; 17, 21 $\times 94$. 1, 2 Stain, Picro carmine; 3-21 Stain, Haemotoxylin.]

FIG. 1. Longitudinal section through wall of a young blastostyle before formation of gonophore. Ova, *ov*, in the endoderm; older ovum towards mouth pole; ova not directly upon supporting lamella. Stain, Picro carmine.

FIG. 2. Longitudinal section through mouth region of a young blastostyle before formation of gonophore. Ovum, *ov*, in endoderm of the tentacles. Stain, Picro carmine.

FIG. 3. Longitudinal section of young female gonophore bud. Breaking down of supporting lamella.

FIG. 4. Longitudinal section of older female gonophore. Ova in endoderm. Bell nucleus consisting of a few cells at distal portion.

FIG. 5. Longitudinal section of adult female gonophore. Ova situated between the endoderm and inner layer of bell nucleus. Bell nucleus consisting of two layers of ectoderm.

FIG. 6. Longitudinal section of distal portion of adult female gonophore, showing a union of the ectoderm and endoderm.

FIG. 7. Longitudinal section of adult female gonophore. Spermatozoa, *sp*, on one side of spadix, *S*; ovum, *ov*, upon opposite side.

FIG. 8. Longitudinal section of young male gonophore bud. Two cells in ectoderm, *a*, *b*, with more granular protoplasm.

FIG. 9. Next section in same series; *a*, *b*, granular cells.

FIG. 10. Longitudinal section of older male gonophore. Bell nucleus consisting of two layers.

FIG. 11. Longitudinal section of still older male gonophore, with spermatozoa, *sp*, situated between the endoderm and outer layer of bell nucleus. Spermatozoa developed from inner layer of bell nucleus.

FIG. 12. Longitudinal section of still older male gonophore. Sexual cells further developed.

FIG. 13. Longitudinal section of adult male gonophore. Spermatozoa, *sp*, fully developed.

PODOCORYNE.

FIG. 14. Longitudinal section of young female medusa-bud of Podocoryne. Ova in endoderm.

FIG. 15. Longitudinal section of a portion of an older medusa-bud, solid plug of ectoderm, *bn*.

FIG. 16. Longitudinal section of manubrium of female medusa, ovum in endoderm; ova passing from endoderm into ectoderm; ova in ectoderm.

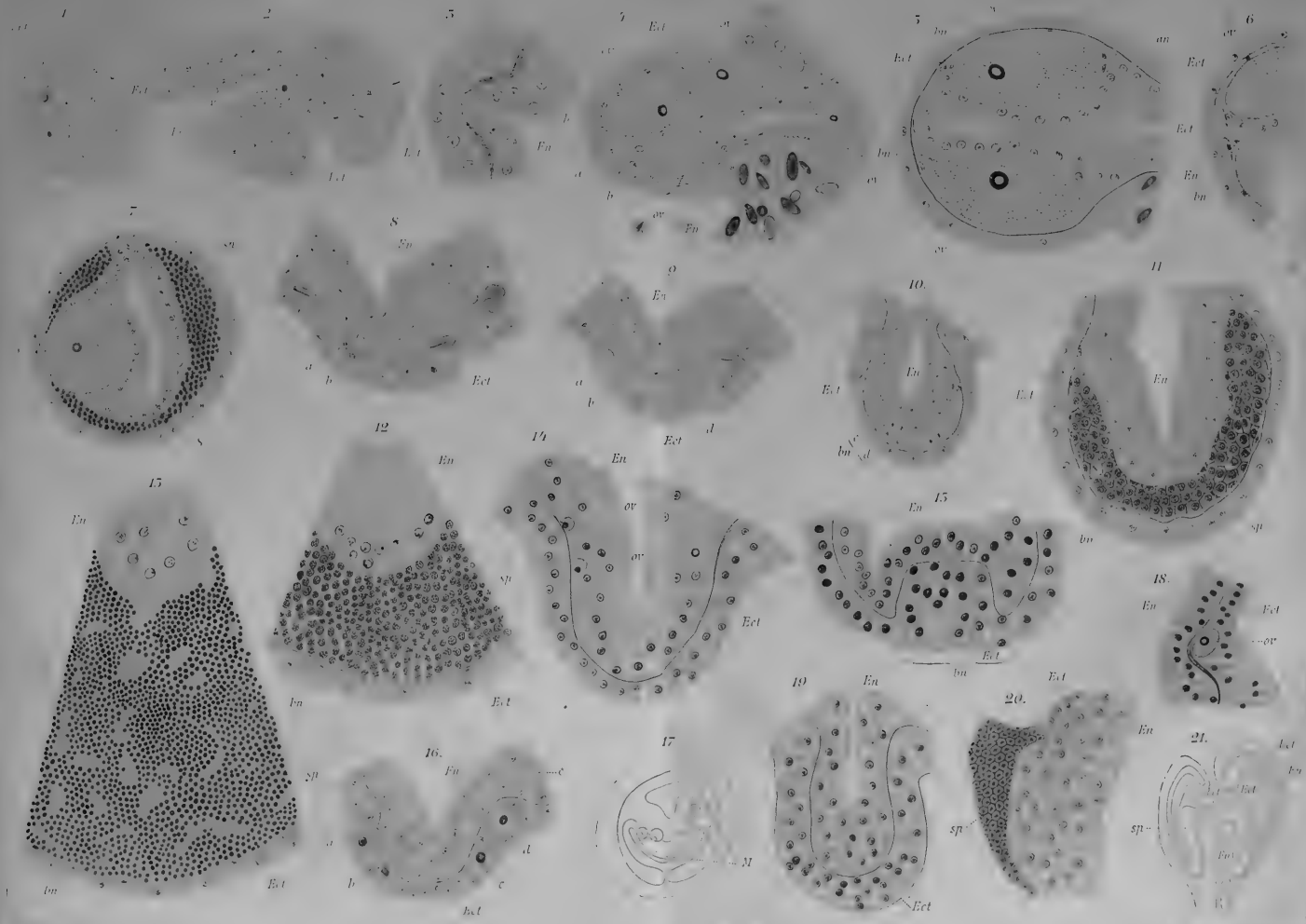
FIG. 17. Outline of female medusa to show orientation of manubrium in Fig. 16.

FIG. 18. Longitudinal section of proximal portion of wall of medusa-bud, ovum in ectoderm.

FIG. 19. Longitudinal section of young male medusa-bud. Many nuclei in distal portion of both ectoderm and endoderm.

FIG. 20. Longitudinal section of portion of manubrium of male medusa, spermatozoa, *sp*.

FIG. 21. Outline drawing of male medusa to show orientation of manubrium in Fig. 20.



EXPLANATION OF PLATE II.

[Figs. 22-44 \times 94. 45-47 \times 31. All drawn from life, with exception of Figs. 30 and 45.]

FIG. 22. Unsegmented eggs, showing the formation of the polar globules.

FIG. 23. Extrusion of one polar globule, division of the other one.

FIG. 24. Two-celled stage in process of formation. Protoplasmic band at one pole. Primary blastomeres, *A*, *B*. (50 min.)

FIG. 25. View of same ovum. Beginning of second cleavage; cleavage centrifugal. (1 hr.)

FIG. 26. View of same ovum. Second cleavage almost completed; protoplasmic bands. Fainter lines represent the points of contact of cells at the opposite pole. (1 hr. 10 min.)

FIG. 27. Commencement of third cleavage, movement of blastomeres. (1 hr. 20 min.)

FIG. 28. The same egg as in Fig. 26; eight-celled stage. (1 hr. 30 min.)

FIG. 29. The same egg as in Fig. 27; sixteen-celled stage. (1 hr. 35 min.)

FIG. 30. Embryo consisting of a spherical mass of cells. (About 2 hr. 30 min. Drawing from preparation.)

FIG. 31. Planula oval in shape. (10-11 hrs.)

FIG. 32. Ciliated planula. (16-17 hrs.)

FIG. 33. Movement of blastomeres; unusual segmentation; beginning of second cleavage.

FIG. 34. Same egg five minutes later; completion of second cleavage.

FIG. 35. Same egg seventeen minutes later; beginning of third cleavage.

FIG. 36. Abnormal planula.

FIG. 37. Two blastomeres of one ovum; separated by cutting.

FIG. 38. Same two blastomeres five minutes later.

FIG. 39. Blastomere of an ovum shaken apart when two-celled; beginning of first cleavage (properly second cleavage).

FIG. 40. Same half embryo fifteen minutes later.

FIG. 41. Same half embryo fifteen minutes later than in 40.

FIG. 42. Same half embryo twenty minutes later than 41.

FIG. 43. Half planula. (About 18 hrs.)

FIG. 44. Half planula, ciliated. (About three days.)

FIG. 45. Longitudinal section of young blastostyle before formation of gonophore. Glands, *g*, in gastral region; ova, *ov*, in germinal region.

FIG. 46. Outline drawing of male blastostyle with gonophores.

FIG. 47. Outline drawing of female blastostyle with gonophores.

22

25

24

25

26

27

1

h

A

B

A

B

B'

h

B'

A

A'

A'

28

29

30

33

34

A

h

A'

A

A

A

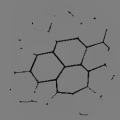
P

P'

B'

38

37



39

B

40

B

A

42

P

A

P'

43

41

35

A

A'

44

32

37

45

43

A

46

h

44

EXPLANATION OF PLATE III.

[Figs. 48-57, 62, 63 \times 187. 58-67 (with exception of 62, 63) \times 94.]

FIG. 48. Section of blastula when about sixteen cells.

FIG. 49. Tenth section of a series of twenty-five.

FIG. 50. Twelfth section of same series.

FIG. 51. Section showing continued multipolar delamination of ectoderm cells to form endoderm. (Embryo about 2 hrs. 40 min. old.)

FIG. 52. Section of embryo about the same age, but further developed. Multipolar delamination. Division of endoderm cells.

FIG. 53. Next section in same series, showing an elongated cell, *a*, delaminating.

FIG. 54. Sixteenth section of series of thirty-nine. Continued multipolar delamination. Elongated cell, *b*. Division of endoderm cells.

FIG. 55. Seventeenth section of same series, showing elongated cell, *b*, with karyokinetic figure.

FIG. 56. Sixteenth section, series of thirty-three. Embryo consisting of solid mass of cells. (About 3 hrs. 30 min.)

FIG. 57. Cross-section of planula (23 hrs.). Formation of coelenteric cavity.

FIG. 58. Longitudinal section of attached planula. ($4\frac{1}{2}$ days.) Coelenteric cavity closed.

FIG. 59. Section of young hydroid. (5 days.) Coelenteric cavity closed. Appearance of two tentacles.

FIG. 60. Section of young hydroid. (About 5 days.) Hydrorhiza, *h*.

FIG. 61. Section of same hydroid, showing mouth.

FIG. 62. Section of half embryo. Karyokinetic figure, *k*.

FIG. 63. Cross-section of half embryo. Breaking down of endoderm to form coelenteric cavity.

FIG. 64. Outline drawing of hydroid about six days old, 3 mm. high (from life). Stolon-like growth at point of attachment. Appearance of two tentacles.

FIG. 65. Outline drawing of hydroid about one week old (from life), viewed from above, showing hydrorhiza, tentacles, and mouth.

FIG. 66. Outline drawing of hydroid about five days old (from preparation). Six tentacles. Hydrorhiza at one point turning up as though to form hydroid.

FIG. 67. Outline drawing of hydroid, nine days old (sketch from life). Budding of hydrorhiza to form reproductive hydroid.

FIG. 68. Medusa of Podocoryne (drawn from life by R. Takano).

48



49



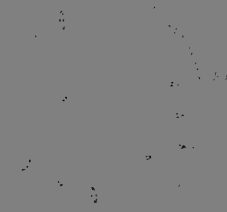
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51



52



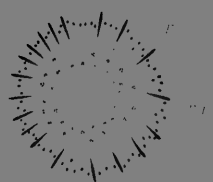
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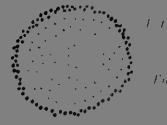
57



58



59



60



61



62



63



THE EPIPHYSIS OF TELEOSTS AND AMIA.¹

CHARLES HILL.

PREVIOUS to 1882 our knowledge of the epiphysis in fishes was limited to the brief notices of Cuvier ('05), Carus ('14), Gottsche ('35), Stannius ('54) and others, who content themselves with references to its existence and macroscopic appearance. Cattie ('82) carefully describes the external features of the organ in adult Teleosts, Plagiostomes and Ganoids, and speaks in some detail of its histological structure. It consists of a granular matrix in which are embedded round, pear-shaped or bipolar cells, the processes of which anastomose with those of neighboring cells.

Rabl-Rückhard ('82) gives a detailed description of the early stages in the development of the epiphysis in *Salmo salar* and *Salmo fario*, and also shows by figures the development of the organ in embryos between 3.5 mm. and 22.5 mm. in length. Its history in embryos longer than 22.5 mm. does not appear to be known.

During the last ten years the attention of morphologists has been turned to the epiphysis in *Petromyzon* and *Reptilia* to the exclusion of nearly all other forms. Hoffmann ('85) in a paper on the development of *Reptilia* records an observation on the development of the epiphysis in *Salmo salar* and *Salmo fario* to which I shall refer in another part of this paper.

This work was begun at the suggestion of Professor J. E. Reighard with the purpose of completing the history of the development of the epiphysis in Teleosts, and of establishing as accurately as possible its adult histological structure.

¹ Work from the Morphological Laboratory of the University of Michigan, under the direction of Prof. J. E. Reighard, accepted as a thesis for the degree of Master of Science.

In what follows the description of *Salmo* 7 mm. in length is based on the study of living embryos, but has been verified and extended by the examination of sections. In older stages the presence of pigment prevented a study of the living embryo, and the descriptions are therefore based altogether on sections. The description of the organ in other Telosts is taken from living embryos, while in *Amia* it is again based exclusively on sections.

As a fixing reagent, Flemming's stronger solution was found to give satisfactory histological results. In older stages, in order to secure a better penetration of the fixing fluid and in order to overcome the difficulty of cutting the lens, the lateral eyes were removed and only the dorsal part of the head immersed in Flemming's fluid. This part was subsequently stained in toto in carminic acid and then sectioned in paraffin. In order to give the nerve fibres a deeper stain the sections, after being fixed on the slide, were immersed for twenty-four hours in saturated carminic acid in 80% alcohol kept at a temperature of 45° C.

In the study of living embryos the egg membranes were always removed. A piece of fine copper wire about two inches in length was twisted into a loop, the diameter of which was a little less than the diameter of the yolk sac of the embryo. The ends of the wire were then bent in such a way that they formed a support for the loop so that when placed in a flat dish filled with water the loop was raised 1 or 2 mm. from the bottom of the dish. The embryo was taken into a wide-mouthed pipette, held in a vertical position, and forced into the loop tail foremost in such a way that the loop, by constricting the yolk sac, held the embryo firmly in position. By bending the wire supporting the loop the embryo could be brought into any desired position, and any view of the head could be obtained.

I desire here to acknowledge my indebtedness to the United States Commissioner of Fish and Fisheries who on several occasions has had sent to me a very liberal supply of *Salmo* material. I am under equal obligations to the Michigan Fish Commission for a supply of *Coregonus* and *Stizostedion* eggs.

SALMO.¹

Salmo 7 mm. long. — In Salmonidae, as in all other vertebrates, the epiphysis has its origin in the roof of the primary fore-brain just cephalad of the posterior commissure. Two small dorsal evaginations of this brain-roof may be found in the embryos of all the species of *Salmo* that I have studied. In *Coregonus albus* I ('91) have designated them as the posterior and the anterior epiphysial vesicles, and I shall use the same words for the corresponding structures of *Salmo*.

Posterior epiphysial vesicle. — In *Salmo 7 mm. long*, the posterior epiphysial vesicle (Figs. 1, 2, 3 and 4, *E*) is a small, nearly globular evagination in the posterior part of the roof of the primary fore-brain. In its center there is a cavity, the middle of which lies a trifle to the right of the median line of the brain so that the vesicle is turned a little to the right. This cavity has the form of a compressed funnel with concave lateral surfaces, and opens ventrally by its narrower end into a short passage which is common to it and the cavity of the anterior epiphysial vesicle. The lateral walls of the vesicle are biconvex. In their thickest middle portion they are about twice as thick as the dorsal wall at its middle. The dorsal wall is also biconvex and its outer surface has a lesser radius of curvature than its inner surface. The vesicle is thinnest at the junction of its dorsal and lateral walls.

Anterior epiphysial vesicle. — The anterior epiphysial vesicle is a club-shaped or ob-ovoid body (Figs. 1, 2, 3 and 4, *E'*). It lies to the left of the median plane of the brain and close against the posterior vesicle. Its bulk may be estimated at two-thirds that of the anterior vesicle. Its distal end is bent slightly caudad, and its long axis is directed laterad and dorsad so as to form with the dorso-ventral diameter of the posterior vesicle an angle of about 75 degrees. It lies in such a position that in a profile view its anterior border coincides with that of the posterior vesicle. Along its middle there is a narrow cavity which curves in conformity with the anterior border of

¹ *Salmo fontinalis* Mitch. *S. purpuratus*, Pallas, and *S. fario* L.

the vesicle, and opens into the common passage which leads from the brain cavity. This passage, which is median, rises for a distance of .021 mm. and then bifurcates, one branch passing to the right to form the cavity of the posterior vesicle and the other to the left to form the cavity of the anterior vesicle (Fig. 4).

As may be seen in a transverse section the left wall of the posterior vesicle passes directly into the right or dorsal wall of the anterior vesicle, while the right wall of the posterior vesicle and the left wall of the anterior vesicle are each continuous laterally with the brain-roof (Fig. 4). Thus the two vesicles are attached to the brain-roof by a common stalk. These vesicles may have been formed in any one of three ways: (1) They may be thought of as independent and separate outgrowths which have subsequently come to be borne on a common evagination of the brain-roof; (2) it is possible to think of them as formed by the division of an originally single vesicle; (3) it is possible to think of the anterior one as formed by constriction from the posterior. It was, perhaps, under the influence of the latter point of view that Strahl and Martin and Béraneck have described the anterior vesicle of *Lacertilia* as formed at the expense of the posterior. I have not obtained satisfactory sections of embryos younger than that which yielded the section shown in Fig. 4, and if one judges by this figure alone there is little to choose between the three methods of origin mentioned above. In Fig. 3a there is shown a view of the roof of the fore-brain in an embryo thirty-seven days old, and in this the two epiphysial vesicles appear as slight and entirely independent elevations of the outer surface of the brain-wall. Beneath each of these elevations is seen a slit-like lumen leading from the brain-cavity toward the outer surface of the brain-wall. These lumina are entirely separate from one another. Unfortunately this observation was made on but one living individual and, owing probably to the rapidity with which the stage is passed through, I was unable to verify it on others.

The brain-roof at the point of union with the stalk of the two vesicles is very much thinner than the adjacent brain-

wall, so that the two epiphysial evaginations rest in a shallow depression of this roof. The epithelium dorsal to the vesicles consists of a single layer of flattened cells (Fig. 4). Between this layer and the brain mesenchyme is always present except just dorsal to the epiphysial vesicles. A section through the epiphysial vesicles parallel to a plane through the middle of the lens of the left eye and through the posterior border of the lens of the right eye passes through the cavities of both vesicles (Fig. 4). Such a section makes an angle of ten degrees with a transverse line.

At a distance of .184 mm. in front of the passage common to the two vesicles there is a small transverse fold of the brain-roof which extends in a postero-ventral direction into the cavity of the primary fore-brain in such a way that the brain-roof between it and the epiphysial vesicles has the appearance of having been evaginated in a dorso-cephalic direction (Fig. 2). Rabl-Rückhard has indicated this fold as the boundary between the prosencephalon and the thalamencephalon. Again at a distance .122 mm. caudad of this passage there is a similar fold accompanied by a thickening of the brain-roof. This is the beginning of the posterior commissure.

The two epiphysial vesicles are encircled by a blood-vessel which passes transversely across the roof of the brain and in which the blood in some embryos circulates from right to left, and in others from left to right (Fig. 3).

The histological structure of the two epiphysial vesicles at this stage of development is essentially the same as that of the adjacent brain-roof. The nuclei are ovoid and so arranged that their long axes are radial to the cavity. The walls of the vesicles are usually two cells deep, except the lateral walls of the posterior one, which are more than two cells deep (Fig. 4).

Since the posterior vesicle becomes the epiphysis of other writers, I shall in the following stages speak of it as such.

Salmo 13 mm. long (92 days old).—*The epiphysis* of *Salmo* 13 mm. long has grown forward. Its median plane coincides with the median plane of the body. Its long axis is curved and its distal portion approaches to parallelism with the axis of the body, so that what was formerly the anterior wall of the

vesicle has become its ventral wall (Fig. 11, *E*). Two parts may be distinguished, a distal enlarged portion and a proximal, narrow, cylindrical portion which connects the distal portion with the brain-roof. The distal part is flattened dorso-ventrally, and lies nearly in a horizontal position, with its distal end very close to the epidermis. Its dorsal surface is convex, while its ventral surface is nearly plane and is closely applied to the roof of the brain. The cavity of the epiphysis is reduced to a narrow cleft which is parallel with its dorsal surface so that the posterior and dorsal walls are of nearly uniform thickness. The ventral wall of the distal part is, on the contrary, three or four times as thick as the anterior wall of the proximal part with which it is continuous.

The anterior epiphysial vesicle remains connected with the brain-roof for a period of only ten days, and shows from the first a less vigorous growth than the epiphysis (Fig. 12). In embryos 13 mm. long its connection with the brain is severed, and the vesicle is wedged in between the walls of the thalamencephalon and mesencephalon at some distance beneath the integument. It lies posterior to the superior commissure against the left wall of the stalk of the epiphysis. A median longitudinal section of the head which passes through the median plane of the posterior vesicle (Fig. 11) does not include the anterior vesicle, but a section $75\ \mu$ to the left of the median plane bisects it (Fig. 12). It is an ovoid body the long axis of which is nearly perpendicular to the dorsal surface of the head. Its anterior and posterior walls are in close contact, so that its cavity is nearly obliterated.

Posterior to the point of union of the epiphysis with the brain-roof is the broad posterior commissure; and in front of this point is the transverse fold, which separates the prosencephalon from the thalamencephalon (Fig. 11 *P* and *K*). This fold is made up of a single layer of columnar cells, and projects into the cavity of the brain in a direction nearly parallel with the long axis of the epiphysis. The anterior part of the roof of the thalamencephalon appears, consequently, to have been evaginated dorso-cephalad in such a way that in a median longitudinal section it bears some resemblance in form to the

epiphysis. Its histology, as well as its method of development, is, however, very different from that of the epiphysis, and will be spoken of in detail in another place.

Lying close against the anterior border of the stalk of the epiphysis and in the dorsal part of the brain-roof is a small column of transverse fibres, the superior commissure (Fig. 11, S). Gottsche ('35, p. 455) speaks of this commissure in Teleosts as *commissura tenuissima*. Stannius ('53, p. 129) finds it in *Pleuronectidae*, but fails to find it in *Cottus scorpius*, while Rabl-Rückhard ('82) in his work on *Salmo* does not mention it. Ahlborn ('83) describes it in *Petromyzon*; Balfour ('78) finds it in *Selachians*. Osborn ('88) finds it in *Amphibia* and calls it superior commissure. The fibres of this commissure may be traced on either side to the structures described by Rabl-Rückhard as probably homologous with the ganglia habenulae of other forms. These fibres are first seen at the time of the formation of the epiphysial vesicles in both *Salmo* and *Coregonus albus*.

The epidermis just dorsal to the epiphysis consists of several layers of columnar cells and of scattered goblet cells opening to the external surface. Between the epidermis and the epiphysis is a plate of undifferentiated tissue into which the distal part of the epiphysis projects so as to lie with its distal end very close to the external epithelium.

Histology.—All the cells of the proximal part of the epiphysis and most of those of the distal part are ovoid and arranged radially to the cavity of the epiphysis. Scattered among the ovoid cells of the distal part are a few cells, the outer ends of which are pointed. In the posterior part of the dorsal wall some of these cells (Fig. 11, *Nrv. Cl.*) are arranged in radial groups, each of which is separated from adjacent groups by a few ovoid cells with ovoid nuclei. The pointed ends of all the cells in each group are approximated and directed toward the outer surface. This grouping of cells is more evident in *Salmo* 25 mm. long, and is described in detail under that head. A few large cells with distinct cell boundaries, and showing certain stages of karyokinesis, are usually found in the distal part of the epiphysis.

The walls of the anterior epiphysial vesicle (Fig. 12) are two or more cells deep. Some of these cells are oval and some are pointed, but the pointed cells are not definitely grouped.

Salmo, 25 mm. long (160 days old). *The Epiphysis*.—In form the epiphysis of *Salmo* 25 mm. long (Fig. 14, *E*) resembles that of the adult animal. It differs from the condition described in *Salmo*, 13 mm. long, principally in the greater elongation of the proximal portion and in its sharper differentiation from the distal portion. The proximal portion makes up about one-half the length of the organ. It is cylindrical, has a transverse diameter of .03 mm. and lies in a position nearly at right angles to the long axis of the body. The distal portion is flattened dorso-ventrally, its transverse axis measures .25 mm., while its dorso-ventral axis measures .11 mm. It is bluntly rounded at its free end and passes insensibly into the proximal portion at its attached end. It extends cephalad from the distal end of the proximal portion, with which it forms an angle of about 110° , so that it is not quite parallel with the long axis of the body. The formation of the cartilaginous roof of the skull between the distal portion of the epiphysis and the epidermis has separated the two by a considerable interval. The ventral surface of the cartilage is excavated for the reception of the dorsal surface of the epiphysis, and the excavation is deepest near its cephalic end.

The dorsal wall of the distal portion, as seen in longitudinal section, is marked on its outer surface at regular intervals by slight excavations, corresponding to which are projections of the inner surface into the central cavity. The whole dorsal wall has thus the appearance of having been thrown into transverse folds. It is necessary to distinguish these apparent folds from the actual folds formed later by the blood-vessels.

Owing to this peculiarity of the dorsal wall the cavity of the distal part is very irregular; it is flattened dorso-ventrally and communicates with the brain cavity below through a narrow passage in the proximal part. This passage, just before opening into the brain cavity, expands like a funnel, and forms what Mihalkovics ('77) has called the recessus infrapinealis.

The anterior epiphysial vesicle has the position, form and histological structure described for the anterior vesicle of *Salmo* 13 mm. long (Fig. 9, *E'*). Usually a longitudinal section of the brain through this vesicle passes also through the distal portion of the epiphysis a little to the left of the median plane. The walls of the anterior vesicle have fused, and the vesicle is thus reduced to a solid mass of cells, wedged in between the thalamencephalon and mesencephalon, and entirely without connection with any surrounding structure.

The integument dorsal to the epiphysis consists of two layers of flattened cells. The cells of the outer layer are parallel to the surface, while those of the inner layer are placed obliquely. Between this integument and the cartilage is a compact layer of tissue which becomes the frontal bones of the adult.

The apparently elevated area of the roof of the thalamencephalon, which lies immediately in front of the epiphysis, has its lateral portions thrown into secondary folds (Fig. 9). These folds project into the thalamocoele, and other similar folds appear in the roof of the thalamencephalon at the sides of the epiphysis. The epiphysis is thus walled in by these folds laterally, by the elevated area of the roof of the thalamencephalon cephalad and by the mid-brain caudad, so that it appears to lie in a median longitudinal depression of the brain roof.

Fibre tracts and nerve-cells are well developed throughout the brain. This is especially true of the anterior portion of the mesencephalon.

Histology.—The cell structure of the proximal part or stalk of the epiphysis is essentially the same as that described in the epiphysial stalk of *Salmo* 13 mm. long. In the distal end of the epiphysis the cells are all oval and have no regular arrangement, while in the rest of the distal portion, and especially in the dorsal wall, most of the cells are pear-shaped. In a horizontal section through the dorsal wall these cells are seen to be arranged in transverse bands (Fig. 13). Each band presents in section two rows of cells separated by a narrow, deeply stained line. This line has a fibrous structure and is

joined by numerous fibrillae, which pass to it from among the neighboring cells. Occasionally one of the fibrillae may be traced from an individual cell to the fibre band. The transverse bands of cells are sinuous, and are separated by lighter areas containing a granular material in which are embedded occasional nuclei. Whether the bands branch or whether they pass without interruption entirely around the epiphysis has not been determined. In a series of longitudinal sections the transverse bands of cells are seen cut across (Fig. 14). The smaller ends of the pear-shaped cells of each band approach one another and are directed toward the surface, so that the cells of each band have a radial arrangement. The pointed end of each cell passes into a nerve fibre which very soon unites with similar fibres from other cells, and these collectively pass in a radial direction to the surface of the epiphysis where they turn to pass along the inner surface of a thin membrane which corresponds to the pia-mater. Some fibres appear to pass posteriorly and others anteriorly. The nerve processes and the cytoplasm take a deeper stain with osmic acid and carminic acid than do the nuclei. The latter are oval, finely granular, and fill nearly the whole cell (Fig. 16). There is a very striking resemblance between these groups of cells and the groups of nerve-cells bordering on the cavity of the mid-brain (Fig. 14). In the two cases the cells are similarly grouped, while the form of each cell, and the direction as well as the appearance of the fibres, is the same. Between the groups of nerve-cells there are usually found a few scattered cells, which resemble the cells in the anterior end of the epiphysis.

The posterior wall of the stalk of the epiphysis is traversed longitudinally by a bundle of very small nerve fibres, which pass posteriorly in the brain-roof to blend finally with the fibres of the posterior commissure (Fig. 14). These fibres take a uniform stain with osmic acid and with carminic acid, and show no trace of the blackening which would indicate the presence of a myelin sheath. Anteriorly, as they approach the distal part of the epiphysis, the fibres diverge and pass close against the inner border of the pia-mater. In serial longitudinal sections, some of them, just before entering the

distal portion, are found to pass to the lateral and ventral walls of the epiphysis. There can be little doubt that all the fibres of this column have their origin (nutritive centers) in nerve-cells situated in the distal part of the epiphysis.

Salmo, 8 cm. long (one year old). — *The epiphysis* of *Salmo* of 8 cm. is much larger than the epiphysis of *Salmo* 25 mm. long, but resembles it in both form and position. Its distal part is flattened dorso-ventrally, and lies dorsal to the cerebrum in a median ventral excavation of the cartilaginous plate (Fig. 15). The principal difference to be noted from the preceding stage concerns the relation of the distal part of the epiphysis to the surrounding blood-vessels. In the preceding stage there had already been formed over the surface of the epiphysis a network of transverse and longitudinal capillaries. In the stage in question the vessels of this network no longer lie on the surface of the epiphysis, but have sunk beneath it. This in-sinking is probably not due to any change in the position of the blood vessels, but to an increase in the size of the epiphysis, which has forced its walls against the capillary network. The result is that the walls of the epiphysis have been forced into numerous folds, each of which occupies one of the interspaces of the network. The vessels on the other hand lie sunken between these folds and at a distance from the surface of the epiphysis (Fig. 15). These vessels never pass through the walls of the epiphysis, nor do they penetrate between its cells, but lie between the folds, close against its external surface. Many of the radiating bands of nerve-cells, which in an earlier stage abutted by their outer ends upon the free surface of the epiphysis, have now been carried inward by the folding of the walls so that they abut upon blood-vessels between the folds.

The anterior epiphysial vesicle lies posterior to the superior commissure between the walls of the thalamencephalon and the mesencephalon, and against the left side of the stalk of the epiphysis. It is an ovoid, compact mass of cells and is sometimes very difficult to differentiate from the surrounding structures. It is a little larger than the corresponding structure in *Salmo* 25 mm. long, but otherwise has undergone no change.

Dorsal to the epiphysis between the integument and the cartilaginous plate the two frontal bones meet in a median suture. Ventral to the distal part of the epiphysis the roof of the thalamencephalon is thrown into a great number of irregular folds (Fig. 15), and is richly supplied with blood-vessels so that it resembles the distal part of the epiphysis. This structure is usually spoken of as the choroid plexus of the third ventricle.

Histology.—The histology of the epiphysis is but little changed from that described in the epiphysis of *Salmo* 25 mm. long. The cell boundaries are less distinct, especially those of the distal end. The transverse bands of radially grouped cells and the fibres of the stalk are more numerous and lie close against each other. These fibres take a light, uniform stain with osmic acid and carminic acid, and show no trace of a myelin sheath. On leaving the stalk they pass posteriorly in a compact bundle along the dorsal part of the brain-roof, and finally blend with the fibres of the posterior commissure (Fig. 10). Just before reaching the distal part of the epiphysis they spread out along the inner border of the pia-mater, and as they pass distally dip down toward the groups of nerve-cells.

The boundaries of the cells of the anterior epiphysial vesicle have become very indistinct, but otherwise I am unable to detect any change from the appearance it presents in *Salmo* 25 mm. long.

Salmo, 16 cm. long (two years old). *The epiphysis.*—The growth of the epiphysis has kept pace with the growth of the head and with the elongation of the latter, the distal portion of the epiphysis has maintained its position dorsal to the cerebrum, where it lies in an excavation of the cartilage. At the same time the angle by which the distal and proximal portions of the epiphysis were formerly united ventrally has gradually been modified into a uniform curve to which the dorsal wall of the epiphysis now conforms (Fig. 15a). The distal part and the distal portion of the stalk retain the cavity, but the proximal portion of the stalk (not shown in the figure) has become solid.

Owing to the further increase in the size of the epiphysis, and owing also to the greater number of blood-vessels on its

surface, the folds present in the preceding stage have deepened, and from them secondary folds have been formed. They have increased in number, depth and complexity, and involve nearly the whole distal part of the epiphysis. The cavity of the epiphysis extends into both primary and secondary folds. In individual sections those portions of the central cavity which extend into the folds often appear to be independent of the central cavity and widely separated from it, but in series of sections it is always possible to trace a connection between the two. Between nearly all the folds are to be found blood-vessels which frequently lie far from the surface, but, just as in *Salmo* one year old, they never lie between its cells. On the contrary, they are always removed from the cavity of the epiphysis a distance equal to the thickness of its walls.

Anterior epiphysial vesicle. — The anterior epiphysial vesicle is present as a small mass of cells occupying the same position as that described in younger forms. I have found it in only one individual of this age, and in this its size was about two-thirds that of the anterior epiphysial vesicle of the yearling fish. Its structure was unchanged.

Histology. — It is convenient to distinguish four regions in the epiphysis at this stage. These are: 1. The distal one-fourth of the distal portion of the epiphysis. 2. The remainder of the distal portion. 3. The distal part of the stalk. 4. The proximal end of the stalk. The regions pass into one another by insensible gradations but differ in their histological characters.

The first region has retained its embryonic structure. The wall is here made up of about two layers of round cells uniformly distributed in a granular matrix, and is neither folded nor penetrated by blood-vessels.

The second region. — As one passes from the first part of the epiphysis toward the stalk the folds of the wall and their accompanying blood-vessels gradually make their appearance and increase in depth and complexity to about the middle of the length of the distal portion. The folds then become again less pronounced and finally disappear at the distal end of the stalk. In this part of the distal portion are found the bands

of radially grouped cells. Each group is now made up of a larger number of cells than in the preceding stage, and is consequently larger. Many of the groups project for a considerable distance into the cavity of the epiphysis. The cells composing the groups are pear-shaped, and are held together in a bundle by their nerve processes. Fig. 16 is a camera-drawing of such a group of nerve-cells as seen in a longitudinal section at the point marked X in Fig. 15*a*. In order not to confuse the figure only a few have been drawn of the large number of cells that could be seen in the section.

If the group is a deep-seated one, its fibres may abut upon the walls of a blood-vessel between two of the folds; if the group is superficial, its fibres may reach the free surface of a fold and pass along the pia-mater. The fibres of each group may pass to the surface independently, or those of two or more adjacent groups may join into a single band, which passes to the surface. If the fibres reach the wall of a blood-vessel, they are seen to turn in a curve and follow the course of the vessel. Individual fibres soon become so intimately associated with the wall of the vessel, that it is not possible to distinguish them from it. These fibres probably follow the blood-vessels to the external surface to join the column of fibres in the stalk.

The third region: In the distal part of the stalk there are no folds and no blood-vessels. The projection into the cavity of the stalk of the groups of nerve-cells gives its walls the appearance of being folded; but this appearance should be distinguished from the actual folds which occur in the distal part of the epiphysis. The fibres coming from the groups of cells in the dorsal wall of the stalk all turn toward the brain, and pass directly into the column of fibres which traverses the stalk. In this respect they appear to differ from the fibres in the distal part of the epiphysis; but it is likely that in all stages the fibres from each group of cells pass in one direction, *i.e.*, toward the brain. The appearance which is sometimes presented of fibres passing in both directions does not probably correspond to the reality. It is more likely that it is to be explained by the fact that the fibres of each bundle separate from one another as the bundle approaches the pia-mater, in

order that each fibre may execute a curve which shall bring it at once and independently in contact with the pia-mater. At least, in every case in which (as in Fig. 16) one can trace the fibres individually for some little distance after they leave the bundle, they are seen to pass toward the brain.

In the distal ends of many of the groups of cells seen in the ventral wall of the stalk are found spherical cavities, one in each group, filled with a slightly granular colloid-like mass (Fig. 15*a*, mt. col.). Such groups of cells are near the central cavity, and their cells have not been seen to give off nerve fibres. A few of them are found also in the distal part of the epiphysis on the ventral side. It is possible that we have here to do with a process of degeneration.

The fourth region: The groups of nerve-cells gradually disappear in the proximal part of the stalk, and are replaced by cells similar to those in the distal end of the epiphysis. A few of the same cells are to be found scattered between the groups of nerve-cells throughout the organ.

Along the dorsal wall of the whole stalk there is a bundle of fine nerve fibres which passes to the posterior commissure. These fibres at first sight appear to have the double contour which is common to medulated fibres, but on careful examination it is found that this appearance is caused by two parallel fibres running close together, and that what seems to be the axis cylinder is merely the line of contact between these fibres. Cross-sections fail to show any myelin sheath, so that these fibres probably never pass beyond the condition of embryonic nerve-fibres. As these fibres are traced into the epiphysis, many are seen to be continuous with the nerve-cells in the dorsal wall of the stalk. Although they are collected into a compact bundle in the proximal end of the stalk, they separate from one another distally, and are difficult to follow.

CATOSTOMUS.

In *Catostomus teres*, Mitch., 8 mm. long (25 days old), Fig. 5, the epiphysial vesicles have the position and form described for *Salmo*, 7 mm. long, and it is therefore unneces-

sary here to enter into a detailed description. The two vesicles lie nearly in a transverse plane, and appear to rise from a common median point in the posterior part of the roof of the primary fore-brain. The cavity of each passes into a common median passage which opens into the brain cavity below. The bulk of the anterior vesicle is less than one-sixth that of the posterior vesicle, and it is therefore not easy to find it.

STIZOSTEDION.

In *Stizostedion vitreum*, Mitch., 5 mm. long (10 days old), the posterior epiphysial vesicle is a small cone-shaped body which is attached to the posterior part of the primary fore-brain by a short, narrow stalk (Fig. 6). It lies in the median plane, with its distal end close against the epidermis of the head. In the living embryo I was unable to detect a cavity in this vesicle.

The anterior epiphysial vesicle is an obovoid body which lies just in front of the posterior vesicle and a little to the left of the median plane (Figs. 6 and 7). Its position corresponds very closely to the position of the anterior epiphysial vesicle in *Coregonus albus*. Its bulk is less than one-seventh that of the posterior vesicle. In a left profile view it appears to lie between the anterior border of the posterior epiphysial vesicle and the brain-roof, and seems to have lost its connection with the latter. The pointed end is turned ventro-caudad towards the union of the posterior vesicle with the brain (Fig. 6). Because of its very small size, this vesicle is not as readily found as the anterior epiphysial vesicle in *Salmo*.

LEPOMIS.

In *Lepomis pallidus*, 2.5 mm. long (Fig. 8), the two epiphysial vesicles have the same position as in *Stizostedion*. In a dorsal view the anterior one lies just to the left of the median plane, while the posterior one occupies a median position. At this stage of development the anterior vesicle is connected with the

brain-roof, and in a left profile view appears to lie between the latter and the anterior border of the posterior vesicle.

I have made no sections of *Catostomus*, *Stizostedion*, and *Lepomis*, but have worked exclusively on living forms.

AMIA.

In *Amia calva* there are two epiphysial vesicles, just as in *Salmo* and the other Teleosts described. These vesicles arise from a common median point in the posterior part of the primary fore-brain between the posterior and superior commissures. I have studied their development in *Amia* 10 mm. long to *Amia* 15 mm. long, but owing to lack of material I am unable to describe their earliest condition, or to give their subsequent history. My study of *Amia* is confined to sections.

The posterior epiphysial vesicle or epiphysis, in *Amia* 10 mm. long, is an obovoid body which tapers a little towards its distal end. It is attached by a narrow short stalk to the roof of the thalamencephalon in the median plane and between the posterior and the superior commissure (Fig 22). Its middle point lies a little to the right of the median plane, while its distal end is turned caudad and lies dorsal to the posterior commissure. Its dorsal surface is convex and lies close against the integument, while its ventral surface is plane and is applied to the brain-wall. This vesicle contains a narrow cavity which extends nearly to the distal end and which communicates with the brain cavity below. This cavity separates a thick ventral wall, three or four cells deep, from a thin dorsal wall of but a single layer of cells. The boundaries of the cavity are sharply contoured only where it opens into the thalamocoel, and here they diverge to inclose the funnel-shaped recessus infrapinealis.

In *Amia* 13 mm. long the formation of a fold has carried the posterior commissure ventrally into the thalamocoel and the epiphysis has assumed a nearly vertical position. It lies close against the integument with its distal end some distance removed from the posterior commissure. In *Amia* 15 mm. long this separation of the epiphysis from the posterior commissure

is carried still further, and the epiphysis is now turned a little cephalad and has the form of an equilateral triangle (Fig. 19). The posterior commissure not only passes further into the thalamocoel, but the fold which separates the mesencephalon from the thalamencephalon has been carried further into the brain cavity in a caudo-ventral direction. The epiphysis in shifting its position has become bent near its middle, so that while as a whole it is turned cephalad, its distal end is, nevertheless, directed caudad (Fig. 19). Along its middle there is a narrow cavity, the borders of which are very indistinct. This cavity extends from the recessus infrapinealis, and follows the anterior and dorsal borders of the epiphysis, so that it is V shaped with its apex turned cephalad.

Anterior epiphysial vesicle.—The anterior epiphysial vesicle in *Amia* 10 mm. long is an ellipsoid body which lies close against the posterior epiphysial vesicle and to the left of the median plane of the brain. It is united to the brain-roof by means of a very short stalk which is common to it and the posterior vesicle (Fig. 21 *E'*). Its distal end is turned a little cephalad of the common stalk, and its long axis makes an angle of a few degrees with a transverse line. Along its middle there is a small cavity which communicates with the thalamocoel in a manner very similar to that described in the anterior vesicle of *Salmo* 7 mm. long. In *Amia* 13 mm. long this vesicle extends further cephalad so as to lie directly dorsal to the superior commissure and with its long axis nearly parallel to the median plane. It still retains its connection with the brain in a manner similar to that in *Amia* 10 mm. long.

In *Amia* 15 mm. long this connection is severed. By the forward growth of the epiphysis the anterior vesicle comes to lie against its left side. It is an ovoid mass of cells lying in such a position that its long axis is nearly parallel to the median plane of the brain. Along its middle there remain traces of a cavity.

Histology.—The histology of the epiphysial vesicles of the stages just described is the same as that of the adjacent brain-wall. The cells are oval or round and are nearly filled by their

nuclei. Each nucleus is finely granular and has a distinct nucleolus. I could see no indication of the grouping of cells described in the epiphysis of *Salmo* 25 mm. long.

On each side of the epiphysial vesicles the cavity of the third ventricle pushes caudad dorsal to the roof of the mid-brain, and ends just caudad of the epiphysis in a blind sac. The dorsal and mesial walls of these sacs consist of a single layer of cells, and extend almost to a level with the dorsal surface of the epiphysial vesicles, so that the latter appear to lie in a longitudinal median groove of the brain-roof.

The posterior commissure makes its first appearance as a thickening of the brain-roof just posterior to the origin of the epiphysial vesicles. This thickening is due to a lengthening of the cells. The nuclei lie in the ventral ends of the elongated cells, while the dorsal portion of each cell becomes transparent and finely granular (Fig. 22). In the older stages this thickening becomes folded near its middle, and is thus carried ventrally into the thalamocoel (Fig. 19).

Cephalad of the point of union of the epiphysial vesicles with the brain, and at a distance of .087 mm., is a transverse fold of the brain-roof which passes ventrally into the thalamocoel and separates the prosencephalon from the thalamencephalon (Figs. 17, 18 and 22, *K*). In this manner the portion of the roof of the thalamencephalon just caudad to this fold has the appearance of being evaginated in a manner similar to that described in *Salmo*.

Just in front of the fold that separates the prosencephalon from the thalamencephalon is a glove-finger-like median evagination in the brain-roof. It makes its appearance in *Amia* 13 mm. long. The posterior wall of this evagination is a part of the fold that separates the prosencephalon from the thalamencephalon (Fig. 18). The anterior wall is a portion of the brain-roof between the two lobes of the cerebrum, while the lateral walls form a part of two invaginations which pass ventrally and laterally into the cavity of the cerebrum (Fig. 20). In *Amia* 15 mm. long this evagination becomes constricted near its union with the brain, and in this manner it approaches the form of an elongated vesicle (Figs. 18 and 20). It is

directed dorso-cephalad, and appears to recede from the ectoblast and pass ventrally with the transverse fold that separates the prosencephalon from the thalamencephalon.

Its walls are identical in structure with the adjacent brain-roof, and consist of a single layer of columnar cells with numerous fine cilia on their ventral ends. In position and method of growth this structure seems to correspond to Selenka's paraphysis. In *Amia* 15 mm. long there is nothing in the histological structure to distinguish it from a simple fold in the brain-roof. It may be thought of as an isolated portion of the roof of the fore-brain which owes its existence to the formation of the folds marked Pl. chr. in Fig. 20, and which are themselves the representatives of the choroid plexuses of the lateral ventricles. These fold into the cavity of the fore-brain, and are continuous on each side of the median plane with the previously formed fold which separates fore-brain from 'tween-brain. They are also continuous with one another in the median line in front. The portion of the fore-brain roof included between them, and thus isolated and apparently elevated through no activity of its own, is the structure marked "Par." in Figs. 18 and 20. Its absence in Teleosts is correlated with the absence of a choroid plexus of the fore-brain. If it is the homologue of the paraphysis, so briefly described by Selenka and Eycleshymer, then, as Eycleshymer has suggested, it may represent an early condition of the "true choroid plexus of the lateral ventricles."

CONCLUSIONS.

During the progress of this work I have examined nearly all of the literature on the epiphysis in every class of vertebrates, but so much still remains to be done on the development of these organs and on their relation to the central nervous system, that the time does not yet seem ripe for an exhaustive criticism. I shall therefore content myself with the examination of a few points more immediately connected with my work.

1. When we compare the account of the development of the epiphysial vesicles in Lacertilia, as given by Béranek ('87), Françotte ('88), Strahl and Martin ('88) and Leydig ('90), with the account as I have given it for Teleosts, we find many points of resemblance.

a. In both Lacertilia and Teleosts the vesicles arise as two small outgrowths in the posterior part of the roof of the primary fore-brain. According to Béranek, Hoffmann and Leydig, the epiphysial vesicles in *Lacerta agilis*, 3 mm. long, communicate with one another, and have a common median opening into the brain-cavity below. This statement is supported by a figure of a longitudinal section through the vesicles, in which the posterior wall of the anterior vesicle is represented as passing directly into the anterior wall of the posterior one, while the anterior wall of the former and the posterior wall of the latter are directly continuous with the brain-roof. (See Béranek, Fig. 9, and Hoffmann, Figs. 1 and 2.) This is the same relation that the epiphysial vesicles bear to one another in Teleosts, as is shown in Figs. 4 and 5 of this paper. These two figures represent nearly transverse sections of *Salmo* and *Catostomus*, and the epiphysial vesicles in these forms therefore lie nearly in a transverse plane. In *Stizostedion*, *Lepomis* and *Coregonus* the anterior vesicle lies cephalad of the posterior one, but to the left of the median plane, and has therefore a position intermediate between the position it has in Lacertilia and the position it has in *Salmo* and *Catostomus*. In the stages earlier than that shown in Figs. 4 and 5, each epiphysial vesicle in *Salmo* probably communicates with the brain cavity by a separate opening, as shown in Fig. 3*a*.

These facts seem to me to indicate that these vesicles arise separately, and that, as they grow in a dorsal direction, they carry a part of the brain-wall with them, and thus form the common median passage which is shown in Figs. 4 and 5. *The only reason for regarding the anterior vesicles as formed at the expense of the distal end of the posterior vesicle is that it is smaller, and, aside from this single fact, one might with equal force consider the posterior vesicle as formed at the expense of*

the anterior. The primitive mode of origin remains to be determined.

b. The cell structure of the vesicles in the early stages is the same in Teleosts that it is in Lacertilia. In both groups the walls of each vesicle are made up of a number of layers of rounded cells, and are consequently distinguished from the adjacent brain-wall by their greater thickness.

c. If we compare the anterior vesicle in Teleosts with the anterior vesicle in Lacertilia, we find that in both groups it very early loses its connection with the brain-roof. In Lacertilia, after this connection is severed, the vesicle passes cephalad, soon takes the form of an eye, and ultimately comes to lie dorsal to the cerebrum just beneath the integument. In Teleosts it does not pass cephalad, but remains throughout life embedded between the walls of the thalamencephalon and the mesencephalon, close against the stalk of the epiphysis, and never reaches an eye-like structure.

d. If we compare the posterior epiphysial vesicle in the two groups, we find the embryological history identical. In the adults of both groups its distal part is turned cephalad, and is very much enlarged, while its proximal part is narrow and cylindrical. The minute structure of the posterior vesicle or epiphysis has not received from morphologists the attention that has been devoted to the parietal eye. Hoffmann ('88) says that cylindrical cells, nerve-fibres and ganglion cells are present in the epiphysis of Hatteria, while Leydig ('90) finds (in Lacertilia) an inner row of columnar cells, and outside of this several layers of round nuclei without distinct cell boundaries. The two sorts of cells recognized by Hoffmann and Leydig suggest a comparison with the nerve-cells and non-nervous cells of the Teleost epiphysis. There is a further resemblance in the fact that in both Teleosts and Lacertilia the epiphysis takes on intimate relations to the blood-vessels.

In view of the resemblance noted above, it seems to me that the two epiphysial vesicles in Teleosts are homologous to the corresponding structures in Lacertilia, the anterior vesicle to the Lacertilian parietal eye, and the posterior vesicle to the Lacertilian epiphysis. If this is true, then the probability that in

Salmo the vesicles are separate outgrowths strengthens somewhat Leydig's ('90) view that they are separate and independent outgrowths in *Lacertilia*.¹

We should then interpret the position of the anterior vesicle of *Stizostedion* and *Lepomis* (in front and to the left of the posterior one) as representing a condition intermediate between that in *Salmo* (at the side of the posterior one) and that in *Lacertilia* (in front of the posterior one). In *Anguis fragilis* the position of the anterior vesicle, described by Strahl and Martin and Hoffmann as not in the median plane with the epiphysis, may also be interpreted as representing a stage intermediate between *Salmo* and *Lacerta*. If we regard the position in *Salmo* as the primitive one, some shifting must have taken place in the other forms. In this shifting the determining factor appears to have been the degree of development of the vesicles. Thus the relatively large size of the anterior vesicle of *Lacertilia*, due to its former functional importance, may have brought it into the median plane, because there was room for it only in that position. The epiphysis in *Lacertilia* may have come to lie in the median plane for the same reason. In Teleosts, on the other hand, the epiphysis alone becomes of considerable size, and the small anterior vesicle finds abundant room in its primitive position at the side of the stalk of the epiphysis. It is only in the early stages in Teleosts, when the two vesicles are large relatively to the brain and are nearly of a size, that crowding causes a partial displacement of both vesicles into the median plane.

If we adopt the alternative hypothesis that the vesicles were originally anterior and posterior, it is difficult to understand

¹ Since the above was written there has appeared a paper by Béraneck (*Sur le nerf pariétal et la morphologie du troisième œil des Vertébrés*, *Anatomischer Anzeiger*, Band VII, Nos. 21 und 22), in which a nerve is described connecting the parietal eye with the brain in embryos of *Anguis* 24-27 mm. long. As this paper goes to press Klinckowström (*Le premier développement de l'œil pineal, l'épiphyse et le nerf pariétal chez Iguana tuberculata*; *Anatomischer Anzeiger*, Band VIII, Nos. 8 und 9) records a similar observation on *Iguana*. Contrary to the conclusions of Klinckowström, these observations seem to me to go a long way toward establishing the independence of the epiphysis and parietal eye, since they indicate that the two structures are supplied by independent nerves which arise from widely separated brain-centers.

the lateral position of the anterior vesicle in Teleosts, and even more difficult to explain the asymmetry found in Anguis.

It seems to me that there is some force in this argument as indicating that the two vesicles were originally situated side by side. If this be granted, it does not necessarily follow that they were primitively paired sense organs, each with its own nerve supply. An accurate knowledge of their connection with the central nervous system, such as might be obtained by a study of larval stages, particularly those of *Petromyzon*, is necessary to a solution of this question.

2. The existence of two epiphyses in *Petromyzon* and many details with regard to them have been made known by the work of Ahlborn ('83), Scott ('87), Owsiannikow ('88), Beard ('89), Gaskell ('90) and Shipley ('87), nevertheless it may be said, in the words used by Scott in speaking of his own work, that there is "no observation which shows the mode of origin of the second epiphysial vesicle." The connection of the two vesicles with the brain also requires further study. An elucidation of these two points will do much to clear up the question of the primitive condition of the vertebrate epiphysis and until it is forthcoming it is useless to speculate on the homology of the Cyclostome epiphyses with those of other vertebrates.¹

3. It is of interest to note that the epiphysis of Teleosts may be compared in its histological structure to the inner layers of the vertebrate retina, the layer of nerve-fibres and the layer of nerve-cells. In the vertebrate retina there is an internal layer of non-medullated nerve-fibres which lie next the vitreous humor and are therefore morphologically superficial to the other elements of the retina. These fibres, or most of them, originate in a layer of ganglion cells (*ganglion nervi optici*) which are deeper than the nerve-fibres and are not in direct continuity with the other cellular elements of the retina. In the Teleosts epiphysis there is also a superficial layer of non-medullated nerve-fibres which arise from a deeper layer of ganglion cells.

¹ Since the completion of this work His has called attention to the importance of this subject. *Zur allgemeinen Morphologie des Gehirns*, *Archiv für Anatomie u. Phys. Ant. Abth.* 1892. — S. 346-384.

SUMMARY.

If the foregoing description of the epiphysis is correct the following facts seem to me to be established :—

1. There are two epiphysial outgrowths from the roof of the primary fore-brain of *Salmo*, *Catostomus* *Stizostedion*, *Lepomis* and *Amia*.

2. These outgrowths form structures that become entirely independent of one another.

3. They are histologically distinct from the choroid plexus.

4. The distal part of the epiphysis contains many nerve-cells arranged in characteristic groups.

5. The nerve-cells in the distal part of the epiphysis are connected with the brain-roof by a tract of nerve-fibres.

6. This tract passes to the posterior commissure.

7. The anterior epiphysial vesicle is rudimentary.

8. There is in *Amia* a structure comparable to the paraphysis of other writers (*Selenka*, *Eycleshymer*).

The following conclusions seem to me warranted by the facts.

9. The anterior epiphysial vesicle of the Teleosts named above and of *Amia* is homologous with the parietal eye of *Lacertilia*.

10. The posterior epiphysial vesicle (epiphysis) of the Teleosts named and of *Amia* is homologous with the epiphysis of *Lacertilia*.

11. It is probable that in their primitive position the two vesicles were side by side.

12. The "paraphysis" of *Amia* may be explained as a portion of the roof of the thalamencephalon, which has been isolated and has taken on a tubular form owing to the formation about it of the choroid plexus.

13. A study of the epiphyses of the larvae of *Petromyzon* with special reference to their connection with the brain is likely to yield results of great importance in settling the question as to whether the epiphyses were paired ancestral sense organs.

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REFERENCE LETTERS.

<i>Car.</i>	Cartilage.	<i>Me. Sut.</i>	Median suture of frontal bones.
<i>Cer.</i>	Cerebrum.	<i>Mt. Col.</i>	Colloid (?) mass.
<i>Cer'bl.</i>	Cerebellum.	<i>Nast. pt.</i>	Nasal pit.
<i>Chd. pl.</i>	Choroid plexes.	<i>N.</i>	Nerve-fibres in stalk of epiphysis.
<i>E.</i>	Posterior epiphysial vesicle.	<i>Nrv. cl.</i>	Nerve-cells in epiphysis.
<i>E'.</i>	Anterior epiphysial vesicle.	<i>Opt. ves.</i>	Optic vesicle.
<i>F.</i>	Primary fore-brain.	<i>P.</i>	Posterior commissure.
<i>Frt. bn.</i>	Frontal bone.	<i>Par.</i>	"Paraphysis."
<i>In.</i>	Integument.	<i>Prs. en.</i>	Prosencephalon.
<i>K.</i>	Boundary between prosencephalon and thalamencephalon.	<i>Re.</i>	Recessus infrapinealis.
<i>Lat. org.</i>	Organ of the lateral line.	<i>S.</i>	Superior commissure.
<i>M.</i>	Mid-brain.	<i>Vs. Sng.</i>	Blood-vessels.
<i>Me.</i>	Medulla.	<i>3 v.</i>	^b Third ventricle.

The figures were all made from camera outlines.

EXPLANATION OF PLATE I.

FIG. 1. Head of *Salmo fontinalis* 7 mm. long, 39 days old. Ventro-cephalic view of the living embryo ($\times 27$).

FIG. 2. Left profile view of the anterior part of *Salmo fontinalis*, 42 days old. Optical section of living embryo ($\times 50$).

FIG. 3. Dorsal view of epiphysial vesicles in *Salmo purpuratus* 7.5 mm. long, 43 days old. From the living embryo ($\times 50$).

FIG. 3a. Transverse optical section of epiphysial vesicles in *Salmo fario*, 37 days old. Living embryo viewed in same position as Fig. 1 ($\times 180$).

FIG. 4. Anterior surface of transverse section through the epiphysial vesicles in *Salmo fontinalis*, 37 days old ($\times 180$).

FIG. 5. Anterior face of a transverse optical section of the epiphysial vesicle in *Catostomus teres*, 8 mm. long, 29 days old. Living embryo viewed in the same position as Fig. 1 ($\times 40$).

FIG. 6. Left profile view of epiphysial vesicles in *Stizostedion vitreum* 5 mm. long, 10 days old. Living embryo ($\times 75$).

FIG. 7. Dorsal view of anterior end of living embryo. Same species and age as Fig. 6 ($\times 60$).

FIG. 8. Left profile view of epiphysial vesicles in *Lepomis pallidus* 2.5 mm. long. Living embryo ($\times 80$).

FIG. 9. Left face of a section from the same series of sections as that from which Fig. 14 is taken. It is the fourth section to the left of the section represented by Fig. 14. The sections are 10μ thick ($\times 90$).

FIG. 10. Longitudinal section of the stalk of the epiphysis of *Salmo fontinalis* 8 cm. long, one year old ($\times 110$).

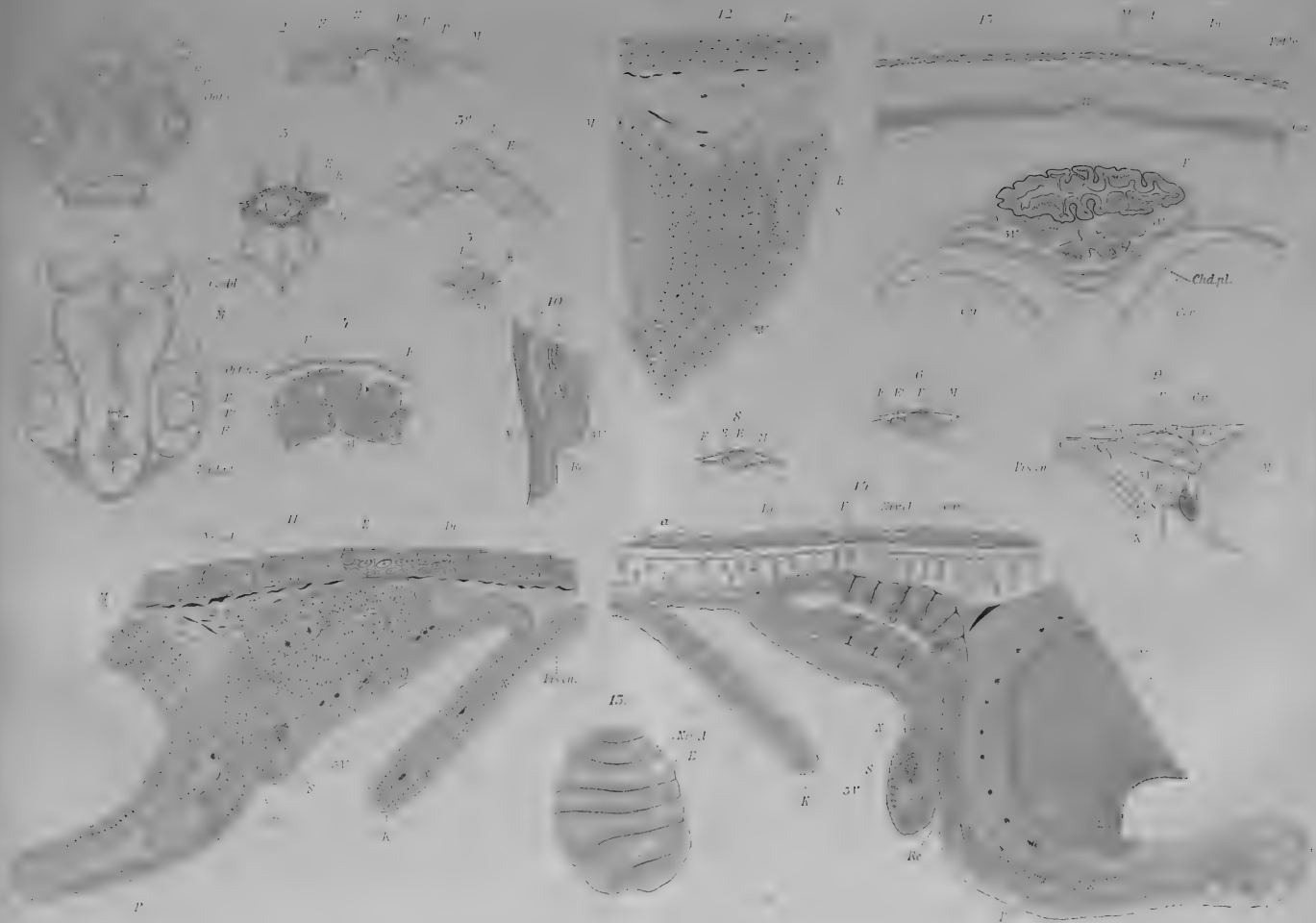
FIG. 11. Right face of a longitudinal median section through the epiphysis of *Salmo fontinalis*, 13 mm. long, 72 days old ($\times 310$).

FIG. 12. From the same series of sections as that in Fig. 11, five sections to the left of the section represented by Fig. 11. The sections are 15μ thick ($\times 310$).

FIG. 13. Frontal section through the posterior part of the dorsal wall of the epiphysis of same species and age as Fig. 14. The section passes along the line *a-b* of Fig. 14 ($\times 180$).

FIG. 14. Left face of a longitudinal median section through the epiphysis of *Salmo purpuratus* 25 mm. long, 160 days old ($\times 180$).

FIG. 15. Anterior face of a transverse section through the distal part of the epiphysis in *Salmo fontinalis* 8 cm. long, one year old ($\times 60$).



EXPLANATION OF PLATE II.

FIG. 15*a*. Left face of a median longitudinal section through the epiphysis of *Salmo purpuratus* 16 cm. long, two years old. About one-third of the epiphysial stalk is shown. X indicates the area represented in Fig. 16 ($\times 75$).

FIG. 16. A group of nerve-cells in the epiphysis of *Salmo purpuratus* 16 cm. long. From the area indicated by the letter X in Fig. 15*a*.

FIG. 17. Section from the same series as Fig. 22. Four sections to the left of that represented by Fig. 22. The sections are 10μ thick ($\times 90$).

FIG. 18. Left face of a longitudinal section through the posterior part of the roof of the prosencephalon and the anterior part of the roof of the thalamencephalon of *Amia calva* 15 mm. long. This section is from the same series as that represented by Fig. 19. Figs. 18 and 19 are so arranged as to represent a single longitudinal section through the roof of the prosencephalon and the thalamencephalon ($\times 75$).

FIG. 19. Left face of a median longitudinal section through the epiphysis and brain-roof in *Amia calva* 15 mm. long ($\times 90$).

FIG. 20. Anterior face of a transverse section through the posterior part of the prosencephalon of *Amia calva* 15 mm. long ($\times 50$).

FIG. 21. Anterior face of a transverse section through the epiphysial vesicles in *Amia calva* 10 mm. long ($\times 180$).

FIG. 22. Right face of a longitudinal median section through the epiphysis and brain-roof of *Amia calva* 10 mm. long ($\times 180$).

Pl. XIII.

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THE PLASTOGAMY OF ACTINOSPHÆRIUM.

HERBERT P. JOHNSON.

IN his valuable and interesting exposition¹ of the phenomena of fertilization, Hartog briefly describes what he considers the most primitive type of the process—the union of two cytoplasmic elements, without accompanying nuclear fusion. This he calls *plastogamy*, and says in regard to it :

“In the true myxomycetes plasmodial fusion always precedes spore-formation. Possibly, as has often been suggested, plasmodial formation has led to the various modes of karyogamy. The nuclei pass freely from place to place in the plasmodium, and may eventually be far removed from what was their original cytoplasm ; and the cytoplasmic elements again undergo a reorganization by their fusion. . . . In this way is fulfilled what I regard as the object also of karyogamy—the association of nucleus and cytoplasm that are strangers to each other.”

What may be termed *accessory* plastogamy necessarily occurs in many kinds of fertilization where the essential factor is unquestionably karyogamy. Instances of this are the formation of a zygote by the union of two zoospores, and the coalescence of the microgamete with the megagamete in the conjugation of Vorticellids. Even among the Metazoa it is maintained by Verworn² that the cytoplasmic as well as the nuclear part of the spermatozoon is not without its influence in the fecundation of the egg.

True plastogamy is characterized by non-fusion of nuclei. It is necessarily a simpler process than karyogamy, and occurring as it does only among the lower Protozoa and Protophyta, may well be regarded as the precursor of nuclear fecundation.

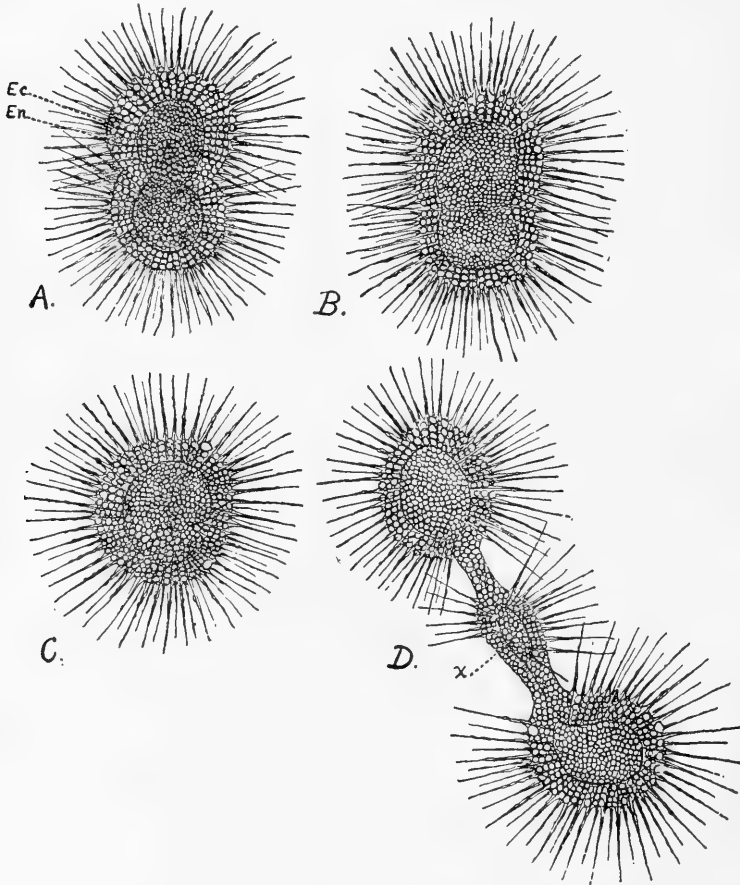
The significance of plastogamy is still very obscure, and it is extremely probable that it is not the same in all cases. The

¹ “Some Problems of Reproduction.” Q. J. M. S., xxxiii, pp. 1-79, 1891.

² Arch. f. d. ges. Physiol., li, p. 98, 1891.

meaning of any particular instance of plastogamy must of course be arrived at, not alone from investigation of the process itself, but from a study of the phenomena preceding and following it.

While at Clark University in the winter of 1891-92, I had an opportunity to study the well-known coalescence of distinct



Series to illustrate the plastogamy and subsequent division of two *Actinosphaeria*. Median optical sections, $\times 27$. Fig. *A*, before the fusion of the endoplasms; Fig. *B*, 45 minutes later; the endoplasms have partially fused; Fig. *C*, completion of plastogamy, 1 hour 5 minutes later than *B*; Fig. *D*, division, 3 hours 45 minutes later than *C*. Division afterwards completed, and the enlarged portion *X* in the commissure became a separate individual. *Ec.*, ectoplasm; *En.*, endoplasm.

individuals of *Actinosphaerium eichhornii*. The specimens were found in a gathering from Lake Quinsigamond, Worcester, Mass., a gathering which had been made two months earlier, and in the meantime had stood undisturbed. Much of the vegetable matter which it contained had decomposed, and an extensive bacterial growth had developed. Early in January *Actinosphaerium* was found in large numbers and of unusual size, many of them exceeding half a millimeter in diameter. Instances of the fusion of two or more individuals were very numerous.

My observations upon the cytoplasmic phenomena of the process do not differ materially from those of Cohn,¹ Gruber,² Penard³ and others upon this form and its near ally, *Actinophrys sol*. Two or more individuals come in contact, and the pseudopodia flow together, forming a sort of network between the gametes, as was described by Cohn⁴ for the present species, and recently by Penard⁵ for *Actinophrys sol*. Then the ectoplasms of the gametes fuse, until at length the endoplasmic central masses are in contact (Fig. *A*). Next, the process involves also the endoplasm (Fig. *B*), till finally the coalescence becomes complete, and the "zygote" is indistinguishable from a normal individual (Fig. *C*). The fusion may take place rather rapidly, as was the case in the series represented in Figs. *A-C* (1 hour, 50 minutes), or may require two or three days. Very frequently it is soon followed by division (Fig. *D*).

The process is not essentially different where more than two individuals fuse; but the greater the number, the more irregular the resultant "colony," and the less perfect the fusion. These colonies usually undergo prompt multiple division, but not necessarily into the original number of individuals. It is not uncommon, for instance, to see a zygote of three individuals divide by a symmetrical constriction into two. In these cases there can be no question of the completeness of the plastogamy.

For study of the nuclei gametes in various stages of coalescence were killed either with picro-acetic mixture or saturated

¹ Z. f. w. Z., iii, p. 66, 1851.

² Z. f. w. Z., xxxviii, p. 62, 1883.

³ Arch. de Biologie, ix, p. 159, 1889.

⁴ Loc. cit., p. 66.

⁵ Loc. cit., p. 159.

aqueous solution of corrosive sublimate heated to about 80° C., stained with Weigert's picro-carmin, and sectioned in paraffine. Hot corrosive sublimate proved a much more satisfactory killing agent than picro-acetic, as far as the preservation of the cytoplasmic reticulum is concerned; but in regard to the nuclei I could see no difference between the two. It was perfectly feasible to embed the specimens in paraffine, and cut unbroken series of sections. In this way it was possible to examine *in situ* every nucleus in the specimen.

All specimens examined contained many nuclei—generally two or three hundred. The greater proportion of the nuclei lie in the cortical portion of the endoplasm as originally described by Max Schultze,¹ but not a few may be found throughout the endoplasm—an observation long ago made also by Greeff.² No nuclei are normally found in the ectoplasm, although a few are sometimes pushed out into it in the course of plastogamic fusion. As is well known from the studies of Gruber³ and of Richard Hertwig,⁴ the nuclei are variable as to structure in different individuals or even in the same animal. They sometimes contain a single large central nucleolus, sometimes two or three of irregular shape, sometimes many. Although scattered nuclei different from the majority may often be found in the same animal, the different types of nuclei—uni- and multinucleolar—are usually characteristic of distinct individuals. The structure of the nuclei has been so fully and accurately described by R. Hertwig⁵ that I need not enter upon further details here.

A striking and significant fact in connection with the plastogamy is the entire absence of mitosis. Mitotic phenomena almost invariably—perhaps always—accompany karyogamy, and it is the rule for nuclei to fuse in some stage of karyokinesis. The work of Gruber⁶ and of R. Hertwig⁷ has shown that the division of nuclei in *Actinosphærium* (which is mitotic)

¹ "Das Protoplasma der Rhizopoden und der Pflanzenzellen," p. 35. Leipzig, 1863.

² Arch. f. mikr. Anat., iii, p. 397, 1867.

³ Z. f. w. Z., xxxviii, p. 374, 1883.

⁴ Jen. Zeitschr., xvii, p. 494, 1884.

⁵ Loc. cit., p. 494.

⁶ Loc. cit., p. 374.

⁷ Loc. cit., p. 491.

is a rare event. Among the many specimens that I have examined I have not found a single instance of it.

Instances of apparent karyogamy are numerous. One frequently finds nuclei in contact, and often the contact is so intimate as to be suggestive of incipient fusion. But the use of a good oil-immersion lens (Zeiss's 3mm. apochromatic) has never failed to reveal a nuclear wall between the two. The nuclei thus seen in contact are by no means always two in number; sometimes four or more are bunched together. But one never finds a giant nucleus, as would of course be the case if complete coalescence of several occurred. Furthermore, nuclei are found in contact, not only at time of plastogamy, but also at time of division, and in the ordinary resting state of the animal. There are, therefore, strong reasons for believing that fusion of nuclei does not take place; at any rate it can have no significance with reference to the plastogamy.

I have stated that coalescence is often followed promptly by division, and the same observation has been made by others (Cohn¹ Cienkowski,² Brandt³). It has often seemed to me that a disturbance amounting to a loss of equilibrium in the vital economy of the organism was induced by plastogamy; for not only is it often followed quickly by division, but frequently by almost amoeboid changes of form. The division in turn may be promptly followed by recoalescence. In one instance, three individuals were found to be slightly adherent. Three and one-half hours later, coalescence was almost complete. Two hours and twenty minutes more, and the zygote was far advanced in binary division. Complete division, however, was not seen. The following morning, sixteen hours later, I found, to my astonishment, that complete recoalescence had taken place. The composite thus formed persisted for two days, once showing a strong tendency to tripartite division, the endoplasm being for a short time separated into three masses. But the actual division did not take place until three days afterward, and was binary.

¹ Loc. cit., p. 67.

² Arch. f. mikr. Anat., i, p. 229, 1865.

³ "Ueber Actinosphærium eichhornii," p. 34, Halle, 1877.

In order to ascertain, if possible, the real significance of the plastogamy in *Actinosphærium*, I isolated conjugates in watch-glasses, and kept a daily record of each culture. For food I used a species of *Bosmina* that was plentiful in the gathering containing the *Actinosphæria*, and furnished almost their sole diet. They were surprisingly successful in capturing these active little crustacea; often two or three would be found embedded in the sarcode of one *Actinosphærium*. Several of the cultures were kept over three months, but none of them showed a change in mode of life. The record of one of the most successful is given herewith:—

Pair coalesced,		5 P.M., Jan. 6.	
2	9.30 A.M.,	" 7.
2	" 8.
2	9.30 A.M.,	" 9.
5	10. A.M.,	" 11.
3 (2 very large, each formed by coalescence of 2)	9.30 A.M.,	" 12.
4	9.30 A.M.,	" 13.
4 (increased in size)	11.20 A.M.,	" 14.
3 (2 coalesced)	12 M.,	" 15.
5 (one very small)	10.10 A.M.,	" 16.
5	11.20 A.M.,	" 18.
6	12 M.,	" 19.
5 (one very small)	12 M.,	" 21.
6	2.45 P.M.,	" 23.
3 (1 + 1 and 1 + 1 + 1)	12 M.,	" 25.
5	12 M.,	" 27.
5	10 A.M.,	" 28.
4 (and one dead)	10.30 A.M.,	" 30.
6	10.30 A.M.,	Feb. 1.
No change in number till Feb. 12.			
4	9.15 A.M.,	" 12.
One isolated	8.15 A.M.,	" 15.
No change in number till Feb. 23.			
2	8.40 A.M.,	" 23.
One isolated.			
1 (in division)	3.40 P.M.,	" 24.
1 (elongated)	5 P.M.,	" 25.
1 "	5 P.M.,	" 26.
1 (normal)	9.30 A.M.,	" 27.
1 "	9 A.M.,	" 29.

1 (elongated)	9.35 A.M., Mar.	2.
2	9.35 A.M., "	3.
2	9 A.M., "	4.
2	9 A.M., "	5.
One isolated.		
1	9 A.M., "	7.
2	9 A.M., "	10.
3	8.35 A.M., "	11.
One isolated ; no change in number till Mar. 31.		
1 (in division)	9.15 A.M., "	30.
2 (one removed)	9.15 A.M., "	31.
1	9.30 A.M., Apr.	1.
No increase in number, and died, Apr. 18.		

It is seen from the record that plastogamy is not followed by encystment, or any notable reproductive activity. But each instance of fusion (*e.g.* Jan. 15, 25) is very likely to be followed by division and considerable increase in number within a day or two.

It has been pointed out by Brandt¹ that coalescence in *Actinosphaerium* does not necessarily follow from mere contact. I have in no instance been able to cause it by bringing individuals together. Artificial coalescence was, however, accomplished by Cienkowski² by amputating a small portion from each individual and bringing the wounded surfaces together. In this way he was able to cause the successive fusion of five individuals to form a compound zygote.

Since it is impossible to ascribe fertilization to the plastogamy, where shall we look for this all-important function in the life-history of *Actinosphaerium*? In Schneider's³ account of the encystment of this species, the statement is made that the cyst-spores ("kugeln") at first contain several nuclei each, but afterwards only one. The presumption is that the nuclei coalesce; but if such be the case, division must subsequently occur, for at the time of escape from the cyst the young *Actinosphaerium* has several nuclei. F. E. Schulze,⁴ on the contrary, found but *one* nucleus in each cyst-spore at all times; and the later observations of Brandt⁵ go to confirm those of Schulze on this point. Brandt, however, found a

¹ Loc. cit., p. 34.² Loc. cit., p. 229.³ Z. f. w. Z., xxi, p. 507.⁴ Arch. f. mikr. Anat., x, p. 345, 1874.⁵ Loc. cit., p. 39.

reduction in the number of nuclei before encystment and spore-formation, but the manner in which this was brought about unfortunately could not be discerned, owing to the increased opacity of the endoplasm. At a later stage in the spore-formation, he says :

“Jedes Theilstück verdichtet sich an seinem Umfange und umgiebt sich mit einer zarten membranartigen Hülle. Innerhalb derselben geht jedes der immer mehr zusammenschrumpfenden Theilstücke eine *Zweitheilung* ein. Die hierdurch entstehenden Hälften enthalten je einen Kern, sind gewöhnlich von der Gestalt eines Kugelsegmentes und liegen zuerst platt zusammen, rücken aber bald etwas auseinander, um sich dann wieder zu nähern und mit einander zu *verschmelzen*.” Here, again, there is uncertainty regarding the fusion of the nuclei.

It will be seen from the foregoing that there is abundant opportunity for karyogamy to take place at time of encystment. It would be fertilization of the closest kind, for the nuclei would have belonged originally to a single cell. But the intimate character of the amphimixia would be largely mitigated by preceding plastogamy, bringing nuclei from different individuals into close propinquity, and thus making possible their subsequent fusion.

In some of my cultures I made the interesting observation that the possibility of plastogamy absolutely determined whether the colony should survive. As already stated, the only food given was *Bosmina*. After repeated divisions, the *Actinosphæria* became so small that they could no longer capture the *Bosminas*, and the only possible escape from starvation was coalescence. In this way the perpetuity of the colony was often assured. It may be claimed that a variety of food would be obtainable under natural conditions, and therefore preclude the occurrence of such an emergency ; but even in my aquaria, where other food was obtainable, I seldom saw any prey taken by *Actinosphærium*, except *Bosmina*.

JOURNAL

OF

MORPHOLOGY.

OBSERVATIONS ON THE GEMMULE AND EGG DEVELOPMENT OF MARINE SPONGES.

HENRY V. WILSON.¹

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¹ Published by permission of Hon. Marshall McDonald, U.S. Commissioner of Fish and Fisheries.

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THE observations described in the present paper were begun more than four years ago, when as Bruce Fellow of the Johns Hopkins University I was permitted by the directors of the fellowship to spend a considerable part of the academic year in the Bahama islands. For their kindness in complying with my wishes in this matter I thank them heartily, and trust they may find in the following pages some justification of that pleasant excursion.

The investigation has been subject to many interruptions. On returning from the Bahamas it was prosecuted for a time in Professor Brooks's laboratory, but other duties incident to my connection with the U.S. Fish Commission interfering, it was laid aside until midsummer, 1890. The Commissioner, Hon. Marshall McDonald, feeling that any enlightened attempts

to be made in the future in the direction of the cultivation of useful sponges would be greatly aided by a knowledge of the life-histories of sponges in general, approved of my wish to continue the investigation, which was made the more attractive by the discovery in a sponge,¹ common about Woods Holl, Mass., of gemmules essentially like those I had already found in a Bahama form. The work was accordingly carried on in the Fish Commission Laboratory at Woods Holl until the fall of 1891, by which time my observations were finished. Realizing that the completed paper would be slow in appearing, I published in the *JOURNAL OF MORPHOLOGY* (Vol. V, No. 3, 1891) a brief account of the more important results.

UNIVERSITY OF NORTH CAROLINA.

I. ADULT STRUCTURE AND GEMMULE DEVELOPMENT OF
ESPERELLA FIBREXILIS, N. SP.

I. ADULT.

Esperella fibrexilis is small, the masses usually having a greatest diameter of 4 or 5 inches. It may assume any shape, sometimes appearing as a flat incrustation, and again as a spheroidal mass. Quite commonly its upper surface forms conical processes, often acute and very ragged. And with these there may be combined irregular ridges with sharply cut edges, as in the sponge shown in Fig. 1. It is rare to find this sponge moderately clean, it being nearly always covered with hydroids, polyzoa, and especially a cylindrical alga, all of which are firmly rooted in the body. The feeble development of the skeleton more than anything else marks it off from the known members of this genus.

Diagnosis. *Esperella fibrexilis*, n. sp. — Sponge amorphous, yellowish-brown, and of slight consistency. Dermal membrane containing no spicules or almost none, everywhere separated from subjacent tissues by subdermal cavities, and everywhere

¹ For calling my attention to this interesting sponge (*Esperella fibrexilis*) I am indebted to Prof. T. H. Morgan.

perforated by closely set pores. Surface appears porous to the eye, owing to abundance of comparatively deep subdermal cavities, which when magnified appear as pore-riddled areas. Between such areas the pores are inconspicuous. Oscula fairly abundant and small, often leading into wide shallow spaces, covered with imperforate membrane. Sponge body consists of network of trabeculae, containing single rows of closely set flagellated chambers. Spicules include smooth oxytylotes of about $\frac{2.5}{100}$ mm. long, toxaspires, sigmaspires, and sigmas $\frac{1.0}{100}$ mm. long, large shovels $\frac{6}{100}$ mm. and small shovels $\frac{3}{100}$ mm. long. The small shovels abundant, but large ones rare; sigmas and sigmaspires abundant, toxaspires less so. Oxytylotes scattered irregularly through the mesoderm, not united into a meshwork. In peripheral region the oxytylotes form radial bundles, which divide into brushes supporting the dermal membrane. In the body of sponge, spicular bundles are few in number and without order in their arrangement. *Wharf piles, Woods Holl, Mass.*

The body of the sponge consists of a network of narrow trabeculae, separated by a system of canals, Pl. XIV, Fig. 2. *Esperella* is one of those sponges in which there is no symmetrical arrangement of parts, and which offer the greatest difficulty to the solution of the question as to what constitutes the typical structure of a sponge individual. For in such a sponge neither the oscula, pores, nor canals, are arranged in such a way as to indicate the division of the body into regions which could be compared with one another, and so be taken as the representatives of individuals.

The canal system belongs to Vosmaer's third type. The distinguishing features of the canal system are that all the canals, afferent as well as efferent, are so wide and spacious. Each chamber has not a special afferent canal of its own, but many chambers are grouped round a single comparatively wide afferent canal into which they open directly. Similarly there is no special efferent canal for each chamber, but many chambers open directly into a wide canal.

The development of numerous wide and for the most part comparatively shallow, afferent canals directly beneath the skin

(the so-called subdermal cavities, *s.d.c.*, Pl. XIV, Fig. 2), separates more or less completely the superficial layer of the sponge from the rest of the body. This superficial layer (ectoderm + thin layer of mesoderm) is known as the dermal membrane, *d. mem.*, Fig. 2. The dermal membrane is bound to the subjacent part of the body by the mesoderm lying between the subdermal cavities. The cavities are so numerous that this mesoderm takes the form of irregular beams or trabeculae, often being nothing more than slender cords, as in Fig. 4, *mes. b.* The dermal membrane may easily be pulled off, and is then found to be everywhere perforated by closely set pores, Fig. 4 (surface view of dermal membrane, from below). The subdermal cavities into which the pores open are of two kinds, of which the larger and deeper appear as pore areas (often rounded) on the surface. These pore areas are conspicuous when the surface of the sponge is examined with a lens, and the dermal membrane between them seems at first sight not to possess any pores (Fig. 5, view of a small area of the surface). This is owing to the fact that the second kind of subdermal cavity, underlying the apparently aporous portion of the dermal membrane, is very small and shallow. That the dermal membrane does possess pores between the pore areas can easily be shown by scraping the membrane free of the subjacent tissue, when the pores at once come into view (region *a* in Fig. 5). Owing to the great number of pore areas the surface of the sponge acquires a characteristically porous appearance. When examined under a low objective or magnifying glass the surface is further diversified by an irregular meshwork of dark bands, which represent the coarser mesodermic trabeculae connecting the dermal membrane with the body of the sponge (Fig. 5).

The water passing through the pores enters the subdermal cavities, *s.d.c.*, Fig. 2, whence it may pass by afferent canals, *a.f.c.*, to the flagellated chambers. - The flagellated chambers are arranged along the sides of the afferent canals, and open directly into them. At their opposite pole the chambers open in the same way into efferent canals (*ef. c.*). Both afferent and efferent canals are relatively large. There is no great difference in size between the afferent canals

and the smaller efferent, and in the body of the sponge it is not practicable to distinguish them. This arrangement of the canal system brings it about that the sponge body is cut up into narrow trabeculae, in which flagellated chambers are arranged in a single layer.

The larger efferent canals are distinguishable without any trouble (*ef. c.*, Fig. 2). They unite with one another and very often open into spacious cavities, just underneath the dermal membrane (here aporous), which are precisely like the subdermal cavities, only much larger. The membrane covering these oscular cavities is perforated by the oscular opening itself, which thus differs from a pore only in size. In Pl. XIV, Fig. 6, a portion of the surface is represented showing an oscular cavity, *os. c.*, with an osculum, *os.* Looking through the osculum, two efferent canals are shown, *ef. c.* Surrounding the oscular cavity are numbers of the conspicuous subdermal cavities, *s. d. c.* Other and much smaller oscula are found, two of which are shown in Fig. 7, *os.* Such oscula seem to be nothing in the world but the openings of certain subdermal cavities from which the covering pore-membrane has disappeared. The canals into which such oscula open, branch very quickly, as is shown in Fig. 7. The oscula taken together are few in number, and are distributed over the surface with entire irregularity. They are not seated on elevations, and are inconspicuous. Nothing in their nature or surroundings could of itself warrant one in regarding them as a different type of structure from the pores.

The flagellated chambers, *f. c.*, Fig. 2, are spheroidal, and in the normal regions (those unaffected by the formation of gemmules) are closely set. The collared cells have small cell bodies, which stain but slightly, but have very characteristic deeply staining nuclei. The mesoderm in the normal trabeculae is rather scanty. It consists of cells of many shapes and sizes, which however pass one into the other by slight gradations. As common a type as any is the rounded or amoeboid cell with a well-staining body, Pls. XIV and XV, Figs. 8 and 9. The body varies greatly in size, and these cells pass by insensible gradations into delicate spindle-shaped cells in which the body

scarcely stains at all and consists of a mere coating for the nucleus, prolonged at opposite ends into slender protoplasmic processes. Such cells are especially abundant in the mesoderm of the dermal membrane, Fig. 4. The edges of pores and oscula are always provided with such cells, which here are especially long and fibre-like, and serve as a support for the free edge. The mesodermic bands which support the dermal membrane (Fig. 4), and the partition walls found in branching canals (*p. w.* Fig. 7) contain great numbers of these fibre-like cells. The ectoderm and the epithelioid lining of the canals are formed of flat cells.

The disposition of the peripheral skeletal bundles is shown in Fig. 2. The bundles are composed of spicules, such as that shown in Fig. 3 *a* (oxytylotes, Sollas), which project slightly from the surface of the dermal membrane, Fig. 2. Besides forming bundles, the oxytylotes are scattered in abundance through the mesoderm, but are not united into a meshwork. A variation from the ordinary type of spicule is occasionally found, with a head like that shown in Fig. 3 *b*. After boiling in caustic potash, some spicules are always found with the pointed end split as in Fig. 3 *c*, doubtless an effect due to the action of caustic potash. The bow-shaped spicules, Fig. 3 *d* (toxaspires, Sollas), the s-shaped spicules, Fig. 3 *f* (sigma-spines), and the sigmas, Fig. 3 *e*, are all of about the same size, the first form being less abundant than the other two. They are found scattered about in the mesoderm in all parts of the sponge.

The large shovel-shaped spicules, of which a face view is given in Fig. 3'' *a*, and a side view in Fig. 3'' *b*, are rare. Shovels of about half the size, of which Fig. 3' *b* gives a face view and Fig. 3' *c* a side view, are comparatively abundant. When the spicule is viewed more or less from the end, Fig. 3' *a*, it is seen that the shovel shape is an illusion, that the blade of the shovel is not flat, but is a figure of three dimensions. If an oval body should be divided by a transverse plane, passing through a point on the equator and a point on the opposite surface somewhat nearer one of the poles, two parts would be obtained, of which the smaller would roughly correspond in

shape with the blade of the shovel. That this is the shape of the shovel blade is gathered from a comparison of the figures *a*, *b*, *c*, Fig. 3', the difference lying in the fact that the transverse diameter of the blade *ll* is greater than the dorso-ventral diameter, *d. v*. The wall of the oval is thickened all round (compare *b* and *c*), the thickening being greatest near the apex and gradually decreasing towards the equator. A special thickening produces a tooth *t* on the ventral surface of the blade. The handle of the shovel is directly continuous with the dorsal surface of the blade, and at its apex is divided into three small sharp-pointed lobes, one ventral, two lateral. Now a shovel-shaped spicule of this sort is developed from a small sigma such as *d*, Fig. 3'. Small sigmas like this and bow-shaped spicules are the only microscleres (smaller spicules) found in the ciliated larva and in the recently attached sponge. The small sigmas are also present in the mesoderm of the adult, and transitional forms between them and the shovels are found. The sigma appears to develop into the shovel in this way. It increases in length and one of the ventral arms, *v'* in *d*, becomes relatively long while the other *v''* grows shorter. The longer arm and the axis back of it then flatten out, and grow in such a way as to become connected at the sides forming the blade of the shovel. The other half of the axis does not flatten, but remains as the handle, the shorter arm of the original sigma persisting as the ventral lobe of the handle apex, the other two lobes being formed as outgrowths. The larger shovels are no doubt derived from the smaller by the production of lateral notches (*l. n.*, Fig. 3''), which divide the continuous blade of the small shovel into a dorsal (*d. l.*, Fig. 3'') and a ventral lobe (*v. l.*, Fig. 3''), the tooth *t* remaining as a thickening of the ventral lobe. The only other observations I am aware of, on the development of this class of spicules, are contained in Ridley & Dendy's Challenger Report on the Monaxonida (21, p. xx). Their account anticipates mine in the chief point, viz. that the chelae are produced by the gradual alteration of sigmas.

The study of the anatomy of such a sponge as *Esperella* would of itself lead one to homologize pores with oscula, and

efferent with afferent canals. One would also be inclined to believe that the position of the oscula is not determined by any deep-lying (though veiled) division of the sponge body into individuals. The homology between pores and oscula would rest on the absence of any structural difference between them (they differ in size, but the variation in the size of the osculum weakens this argument), and on their similarity in the matter of local surroundings (comp. Figs. 6 and 7—both pores and oscula open into comparatively shallow, spacious cavities strikingly alike). The homology between the two sorts of canals would rest on their entire similarity—there is no discoverable difference between the subdermal cavities into which the pores open, Fig. 7, and the oscular cavities shown in this figure and in Fig. 6. (The development shows also that in this sponge they are formed in precisely the same way.) As to the basis of the third conclusion, the oscula are distributed with entire irregularity, and the oscular cavities cannot be regarded as so many centers round which the canal system of the sponge groups itself. Rather, it would seem from an examination of such portions of the surface as that shown in Fig. 7, that circumstances may determine the transformation of a pore area into an osculum almost anywhere. The comparative anatomy of sponges in general, however, forces upon us the conviction that forms like this are phylogenetically colonies, even though it be true that new oscula may be formed in an individual independently of any process of budding. And, further, we are driven to believe that phylogenetically, afferent and efferent canals are radically different things, the latter being lined with endoderm, while the former are invaginations from the exterior.

Embryological Methods.—If an *Esperella* be examined during the summer months, it is found to contain great numbers of embryos imbedded in the mesoderm. When these embryos are studied they are found not to be egg embryos, but gemmules (*i.e.* internal buds). Nevertheless, the gemmules in sponges kept in aquaria escape through the oscula as ciliated larvae, essentially identical in structure with the typical egg larva of silicious sponges. After swimming about for a day or

two the ciliated larvae attach themselves to the wall of the aquarium, and undergo a metamorphosis.

Esperella, being of small size, is easily kept in aquaria, but the larvae, as a rule, do not escape in great numbers. A confinement over night in a simple aquarium of good size, say three gallons, through which no water is passing, will nearly always result in the liberation of some larvae. Instead of trying to aërate the water, it was found more convenient to transfer the larvae with the help of a pipette to fresh dishes of water. As most of them attach in a day or so, it is only necessary to transfer a few times. Once attached to the wall of the dish, the dish itself may be placed in a running aquarium and the little sponges thus kept without further trouble. I could not, however, succeed in getting them to increase much in size, in spite of the aquarium facilities in the Fish Commission laboratory. They seemed willing to live indefinitely, but grow they would not — for lack of proper food, I suppose.

The young sponge after the metamorphosis is so thin (scarcely more than an incrustation) that it cannot be scraped off the dish without injury to it. I, therefore, coated my dishes with a thin layer of paraffine. Collodion was also used. A little piece of the paraffine, or collodion, could then be cut out with the sponge sticking to it, and the whole thing placed in the killing fluid, and subsequently kept in alcohol until ready for use. In many cases the little sponge separates at once from the paraffine in the killing fluid. I satisfied myself, by comparison with larvae scraped from the dish, that the paraffine or collodion did not affect the character of the tissues. To be sure such larvae as remained stuck to the paraffine were only kept in alcohol for a few weeks. If the action of the alcohol on the paraffine be kept up for months, I am not sure but the effect on the tissues of the sponge is injurious.

For fixing purposes I found very much the best fluid was the mixture of acetic acid, alcohol, and osmic acid, recommended by Zacharias (glacial acetic 1 part, absolute alcohol 4 parts, osmic acid few drops). I allowed this to act 10–20 mins. It is excellent for all stages of the development.

Kleinenberg's picric proved itself of use for special points, often preserving the individual cells in a more natural and uncontracted condition than the Zacharias. But in general it dissociated the elements too much. Borax carmine and haematoxylin stained in a very satisfactory way. For macerating purposes Bela Haller's mixture was chiefly used.

2. FORMATION OF GEMMULES.

Any portion of the sponge body may develop gemmules. They may be found in the extreme peripheral region, visible under the surface of the uninjured sponge, or may be present in the center of the body. In whatever region they are found they are usually so abundant as to greatly change the structure of the sponge body in that district. In many *Esperellas*, during the summer, the whole body seems given over to the formation of gemmules. In such individuals gemmules are thickly scattered through every part, and the organization of the entire sponge is seriously interfered with. (This interference, as will be shown later, consists largely in the reduction in number of the flagellated chambers, in the obliteration of many canals, and the rupturing of trabeculae.) In other individuals the gemmules may be extremely abundant in certain portions of the body, while the normal sponge structure is retained elsewhere. The older gemmules and the larvae are easily seen with the naked eye. All gradations of size are found down to minute gemmules consisting of but a few cells. The older gemmules and larvae project into the larger canals, the younger gemmules lie in the trabeculae imbedded in the mesoderm. In the section Pl. XV, Fig. 12, are shown a young larva, *l*, a full sized gemmule, *g*, medium sized gemmule, *g'*, and several little gemmules, *g''*. In the section Pl. XIV, Fig. 8, four young gemmules of different sizes are shown lying in the mesoderm.

The formation of gemmules in large numbers is associated with a certain degeneration of the normal sponge structure. This is evident when sections through a region in which gemmules are numerous (Pl. XIV, Fig. 8) are compared with sections

through a region in which few or no gemmules appear (Pl. XIV, Fig. 2). In the latter the trabeculae are made up chiefly of rows of flagellated chambers, with but a scanty amount of mesoderm between the chambers. But in the former the flagellated chambers are either absent or are very few in number. The trabeculae in such a region are composed of mesoderm, with gemmules, and a flagellated chamber here and there. What I take to be the remains of degenerated flagellated chambers are scattered about through the mesoderm. Such are the groups of cells, *deg. f. c.*, in Pl. XIV, Fig. 8, and Pl. XV, Fig. 15. The cells composing such groups are quite like the lining cells of the chambers in general appearance, that is, they have a small clear body which stains scarcely at all and the peculiar nucleus of the collared cell. The inference from these data is that where gemmules begin to develop in large numbers, the flagellated chambers of the region degenerate. What becomes of the collared cells I cannot say, but Metschnikoff's observations on the disappearance and reappearance of the flagellated chambers in young spongillas (12) make it probable that these cells are transformed into amoeboid mesoderm cells. Where gemmules are very numerous, the trabeculae themselves are ruptured and broken down in many places. This is the natural result of the compression of the tissues due to the growth of the gemmules, in the course of which many of the neighboring smaller canals are obliterated, and of the liberation of the gemmules. In such spots the sponge body consists of scarcely more than an amorphous aggregate of cells and gemmules, and affords a noticeable appearance of degeneration when compared with the smoothly outlined trabeculae of a non-gemmular district.

Very young gemmules, such as *g'*, Pl. XIV, Fig. 8, and *g'*, Pl. XV, Fig. 9, are composed of a small number of polygonal cells surrounded by a follicle of flattened cells (*g. f.*). I have never found a gemmule surrounded by a follicle to have less than five cells in cross section. The bodies of the gemmule cells are filled with a finely granular yolk, and take the stain well (haematoxylin or carmine). The nuclei are always conspicuous but differ much in appearance, the difference being due, as I think,

to a difference in the stage of division. In young gemmules such as these, and in considerably larger ones as well, the cell outlines are exceedingly plain.

Young gemmules like those just described are formed from groups of mesoderm cells, such as are shown in Pl. XIV, Fig. 8, and Pl. XV, Figs. 13, 14, 15 (*mes. gr.*). The cells composing the mesoderm group are essentially like the gemmule cells. Like the latter they have plump bodies filled with the same finely granular yolk, in consequence of which they stain well, and have conspicuous nuclei. Such groups of mesoderm cells occur in abundance. They have no definite shape and may contain few cells or many, and the component cells may lie together very loosely or be packed pretty closely. They are formed by the migration towards a common point of certain mesoderm cells in which a considerable amount of yolk has been deposited. Such cells are found in abundance lying singly, or in twos and threes through the mesoderm. In Pl. XV, Fig. 13, there are several (*g. m. c.*). They do not form a class by themselves, but are merely ordinary mesoderm cells containing a maximum amount of yolk, and are connected by transitional stages, containing less and less of yolk, with the delicate spindle-shaped mesoderm cells, the body of which contains no yolk and scarcely stains at all. The congregation of such cells to form groups may be inferred from such preparations as those shown in Pl. XV, Figs. 13 and 14.

In the transformation of such masses of mesoderm cells as are shown in Fig. 15 (*mes. gr.*) into gemmules, the outer cells must flatten and become the follicle. But I have not succeeded in getting preparations actually showing this. I do not believe the gemmule, when first formed, is of any particular size, for groups of mesoderm cells are met with, differing greatly in this respect. The great number of very small gemmules such as *g'*, Fig. 8, and *g'*, Fig. 9, make it evident that very frequently gemmules are formed from masses consisting of but a few mesoderm cells, for instance *mes. gr.* in Fig. 8. On the other hand, it seems likely that a mass of cells so rounded as the larger group in Fig. 15 was about to form a single gemmule, which would have been of considerable size.

Bearing in mind the theoretical possibility of a gemmule originating from a single cell, I went to considerable pains in looking for any such indication. I could not convince myself with certainty that a gemmule ever was so formed, though I found cell groups such as *a*, Pl. XV, Figs. 10, 11, 13, looking as though they had been derived from single cells. Such groups though were very rare.

Gemmules increase in size by cell-division. This is inferred at once from the large size of the cells forming the youngest gemmules as compared with the cells of older ones, Fig. 9. No karyokinetic figures were found, but the nucleus appears in several conditions, representing, no doubt, different phases of nuclear division. These different conditions of the nucleus are shown in Fig. 8' (1, 2, 3, 4, 5), the arrangement of the figures indicating what I take to be the order in which the several phases follow one another. In stage 1, in which are all of the nuclei in the gemmules of Fig. 8, the chromatin forms a solid, usually angular, mass, and the nucleus is small. The mass of chromatin is relatively so large that very often it is difficult to make out the surrounding nuclear membrane, and the nucleus appears to be simply an angular mass of chromatin, as in the larger gemmule of Fig. 9. In what I take to be the second stage the nucleus is larger, and the chromatin forms a tangled skein lying in the center of the nuclear cavity. The smaller gemmule in Fig. 11 has its nuclei in this phase. In the third stage the nucleus is large, and the nucleoplasm very conspicuous, the chromatin being distributed all round the periphery. In the fourth stage there is no increase in size, but the chromatin is here collected at opposite poles. Examples of both these stages may be found in Figs. 9 and 11—it is here seen that the several cells of a gemmule may be in very different stages of division. The remaining stage, which I take to be the one resulting from the act of division, is shown in Fig. 8' (5). It is considerably smaller than 3 and 4, and the chromatin is confined to one side of the nucleus where it forms a thin but dense layer. Nuclei in this condition are shown in Pl. XIV, Figs. 13 and 14. These several conditions of the nucleus of the gemmule cell are all abundant and easily

found. Whether or not they follow one another in precisely the sequence I have indicated, it is plain that the nuclear division, though it perhaps cannot be ranked as a karyokinetic one, is something more complex than a simple constriction of nuclear matter into two parts.

Gemmules increase in size not only by means of ordinary growth, but by fusion with one another. I think this is evident from the following facts. It is extremely common for small gemmules to occur in groups. In such groups, Pl. XIV, Fig. 8, and Pl. XV, Fig. 17, the separate follicles are often so closely pressed together as to be indistinguishable one from the other. Instances are met with not infrequently, where the shape of the gemmule gives strong indication that it has been formed by the fusion of separate parts. This is true of the gemmule x in Fig. 17, and still more so of x in Fig. 16. In Fig. 16 the dual origin of the gemmule is further indicated by the fact that one half the gemmule has nearly all its nuclei in one phase, while the other half has its nuclei in a different phase. Fusion is, I think, confined to the smaller gemmules such as those just referred to. I have not met evidence of it in the case of larger gemmules such as that shown in Fig. 19.

The gemmule, increasing in size in these two ways, grows steadily larger. An idea of the amount of increase may be got from a series of figures representing gemmules of successively larger size from quite small ones up to the mature gemmule. Such a series is given in Pl. XV, Figs. 17, 20, 20', 19, 18. It is remarkable that while the small and large gemmules are both very abundant, medium sized ones such as that shown in Fig. 19 are hard to find. As the gemmule increases in size, it undergoes certain other changes as well. The fine yolk contained in the cells becomes more abundant, and the cells in consequence take a somewhat deeper stain. The cells become gradually much more tightly packed together than they were in the younger gemmules, and the cell outlines grow less distinct. The nuclei grow smaller. In the mature gemmule, Pl. XV, Fig. 18, the cells are so full of yolk and so tightly packed that it is very difficult to make out the cell outlines. They appear as cracks in a uniformly granular and deeply staining substance.

The nuclei are so small that one cannot make much out of them. The central chromatin mass is conspicuous and relatively so large that it is only in exceptional cases that the nuclear membrane can be made out. As a rule, all one can see in a section of the mature gemmule, is a number of small chromatin masses scattered through a finely granular and deeply staining matrix. This veiling of the cellular nature of the mature gemmule in *Esperella* is of importance, it will be seen, as explaining the nature of the gemmule in *Tedania*.

The young gemmule, as has been said, lies in the mesoderm of a trabecula. It does not project into the canals, and it is surrounded by a follicle composed of a single layer of flattened cells. Such young gemmules are shown in Pl. XV, Fig. 12, *g''*. As the gemmule grows it compresses the surrounding tissue, and begins to project into one of the adjacent canals. The gemmule *g'* in Fig. 12 may be taken as illustrating this stage. With the increase in growth the gemmule comes eventually to lie in the cavity of a canal, the surrounding tissue having been gradually compressed into the form of a sheath, which is suspended from the wall of the canal by strands of tissue. The larva *l*, and the mature gemmule *g*, of Fig. 12, illustrate this stage. The sheath, *sh.*, Pl. XV, Fig. 12, and Pl. XVI, Figs. 21, 22, consists of several layers of flattened cells and is indistinguishably fused with the original follicle, except in rare places such as that shown in Fig. 22, where inside the sheath is seen, at one end of the gemmule, the original follicle, *g.f.* In the case of this gemmule, Fig. 22, the compression of the surrounding tissue has not involved the mesoderm at one end of the gemmule, and in this region flagellated chambers are still to be seen.

Though the sponge during the summer is filled with gemmules, the asexual breeding season being apparently at its height, small egg-cells are met with here and there. They are not common but can be found after a little search. The egg-cells are always quite small and in the midst of a large collection of mesoderm cells closely packed, Pl. XV, Fig. 20'', *o. ov.* As a rule they have not a follicle, and in this condition are probably amoeboid — witness the process of the ovum in Fig.

20". The egg-cell has always a large nucleus with a very large nucleolus. In the cytoplasm there are usually several deeply staining bodies, each surrounded by a clear space. One of these bodies in Fig. 20" is quite large. The bodies stain as deeply as chromatin, and I suspect them to be the remnants of engulfed cells. The occurrence of such bodies in the cytoplasm coupled with the fact that the outlines of the egg-cell are often indistinct in spots, suggests that the ovum is feeding on the surrounding mesoderm cells. In a very few cases I have met an egg-cell in the peculiar situation illustrated by Pl. XV, Fig. 20". A gemmule, *g*, of about full size, is only partially surrounded by its follicle. The bare portion is continuous with a thickly packed mass of mesoderm cells, in which lies the egg-cell, *o. ov.* Pl. XVI, Fig. 20", is a more highly magnified view of the bare end of the gemmule. The gemmule cells, *g*, fade away into the less densely packed mesoderm cells, in the midst of which is the ovum, *o. ov.*, surrounded by a follicle, *ov. f.*, which was not present in the egg shown in Fig. 20". It seems pretty clear that the gemmule, *g*, after reaching its full size, burst or absorbed its follicle and became continuous with the surrounding mesoderm. Only these very small egg-cells are met with, but they serve to indicate that a sexual breeding season follows the gemmular season.

In this connection I may speak of certain gemmule-like bodies, which I am unable to explain, but which resemble a stage in spermatogenesis more than anything I know of. (See Fiedler's figures for *Spongilla*, 5, and those of Vosmaer for *Leucosolenia*, 33, Taf. xxix.) Two of these problematical bodies are shown in Pl. XV, Fig. 17, *pr. g.*, and another in Pl. XV, Fig. 14. They are comparatively common. They consist of a follicle inside which are small spherical cells entirely free from one another. The substance of these cells, if cells they are, stains feebly and appears homogeneous, and to the outer surface of each clings a crescentic band of chromatin. These bodies are always of small size, like those shown in Figs. 14 and 17. Their size and follicle suggest that they are derived from gemmules. At first I thought they were degenerating gemmules, but their uniform appearance scarcely admits of this

mass becomes more and more compact until the cells of which it is composed acquire an irregularly polygonal shape owing to mutual pressure (Fig. 25). The ectoderm cells covering the pole become more or less cubical, and at this time do not differ in appearance from the subjacent parenchyma. They neither develop pigment nor cilia, and this end of the embryo is therefore sharply marked off from the rest of the body. The remaining part of the parenchyma is made up of amoeboid cells provided with slender processes connected together into a network. The bodies of all these cells are plump and stain well. When the embryo has reached this stage of development (Fig. 25) spicules make their first appearance. They are few in number and mostly the long, slender oxytylotes. Besides the oxytylotes some curved spicules appear, the embryonic representatives of the bow-shaped spicules shown in Fig. 3*d*.

The development proceeds a little farther than the stage shown in Fig. 25, and the embryo is then set free as a ciliated larva which escapes from the body of the parent through an osculum. In Pl. XVI, Fig. 26, a surface figure of this larva is given, and in Pl. XVI, Fig. 29, a longitudinal section. The greater part of the body is of a deep orange color but the posterior pole (*p.p.*) is unpigmented. The line of separation between the two regions is a perfectly sharp one. The posterior pole ends in a pointed protuberance (Fig. 26) which appears to be a specific characteristic. A bundle of straight spicules (oxytylotes) is conspicuous in this end of the larva. In its general appearance and motion the larva is very like a coelenterate planula. Like the latter it may swim freely through the water, or may creep worm-like over the bottom and sides of the dish, the pigmented pole being posterior.

The cells of the ciliated ectoderm are very long and slender and the nuclei are packed closely in several tiers, so as to form a very conspicuous zone in sections (Fig. 29). The arrangement of several ectoderm cells is shown in the maceration preparation, Pl. XVI, *b*, Fig. 31, and one of the cells more highly magnified in *a* of same figure. There is a single flagellum to each cell. In the peripheral end of the cell is deposited the orange pigment, in the shape of small rounded masses (*p.a.*).

There then follows a clear area (*c.a.*) in which no large granules are found. A coarsely granular region (*g.a.*) comes next. The nucleus is always at the lower end of the cell, which terminates in a delicate process. The ectoderm cells over the posterior pole are of the sort shown in Pl. XVII, Fig. 33, which represents a maceration preparation of this region. The bodies of the cells extend down in an irregular fashion into the mass of parenchyma, and they take a deep stain with haematoxylin. The transition from them to the ciliated and columnar ectoderm is an abrupt one, as may be seen in the section, Fig. 29.

The parenchyma of the swimming larva is considerably more differentiated than in Fig. 25. The cells in the posterior part of the body, Fig. 29, are closely packed and polygonal. They stain feebly and their cell outlines are indistinct. In front of these cells and about in the middle of the body, is a region containing a large number of cells with plump, finely granular bodies, taking the stain well. These cells are of special importance in building up the internal tissues of the sponge and may be spoken of as formative cells. The formative cells are rounded or amoeboid in shape, with slender processes which connect the cells together. In Pl. XVI, Fig. 32, a group of such cells, as seen in a maceration preparation, is shown. The anterior part of the larva is largely occupied by fusiform cells with small bodies, taking the stain very feebly, and terminating at each end in a slender process. Scattered here and there amongst the fusiform cells are a few well-staining granular cells. It is probable from the structure of the earlier larva as well as the later, that all the cells in this stage are connected together by processes. But in macerations this was only clearly brought out in the case of the formative cells. The direction of the fusiform cells round the periphery (Fig. 29) probably indicates a connection between them and the slender terminal processes of the ectoderm cells, Fig. 31.

In the swimming larva there are three kinds of spicules present. Imbedded in the mass of pale polygonal cells of the posterior end are a number of straight spicules (oxytylotes) arranged in a loose bundle with their sharp ends pointing towards the posterior pole. These spicules very often are

found with a little mass of protoplasm and a nucleus sticking to one side, but I could come to no conclusion as to their mode of formation. The bow-shaped spicules, mentioned as present in the earlier stage, are now found in greater number, but still there are only a few of them. In the hollow of the bow there is an accumulation of protoplasm with a nucleus, and the indications are that the spicule is formed as a superficial secretion of this mass of protoplasm. The bow-shaped spicules are almost all found in the posterior half of the larva. The same is true of the third kind of spicule, the rosettes of embryonic shovels. No rosettes are shown in Fig. 29, but in Fig. 30 there are three shown, and Pl. XVII, Fig. 34, represents such a rosette (seen in section) more highly magnified. The spicules are very thin and delicate as well as small, and are not (at this time) found separately, but always united in rosettes. The rosettes are few in number and are usually found close under the ectoderm at the posterior pole.

4. METAMORPHOSIS.

The ciliated larva swims freely for a day or two. As a rule, some time during the second day after birth, it sinks to the bottom and begins to attach. The first step in the metamorphosis takes place while the larva is still swimming freely about. This consists in the flattening of the ectoderm. Pl. XVI, Fig. 27, represents a surface view of a larva 36 hours after birth, and Fig. 30 a longitudinal section of the same stage. On comparing these figures with the corresponding figures made from a larva just hatched (Figs. 26 and 29), it will be seen that the posterior unpigmented area, or region of flat ectoderm cells, has increased in extent at the expense of the pigmented area or region of columnar cells. By keeping the same larva under observation, it can be seen that the unpigmented area gradually extends forwards. As I have said, the process begins while the larva is swimming freely about. It continues after the larva has sunk to the bottom. Pl. XVI, Fig. 28, shows a surface view of a larva in course of attachment. In this larva the pigmented region is reduced to a small area at

the non-spicular pole, and this area will gradually disappear, the disappearance taking place from the spicular pole forwards. Though I have not actually witnessed the transformation of columnar cells into flat cells, this is undoubtedly what takes place. Close observation fails to reveal the casting off of any portion of the larval ectoderm, and sections give every indication that the columnar ectoderm is gradually transformed into a covering of flat unciliated cells. The replacement of columnar cells by flat ones never fails to take place in the manner described, *i.e.*, gradually from the spicular pole forwards. Now the surface area of one of the flat cells is considerably greater than that of a columnar cell and since the entire area to be covered remains approximately the same, it is obvious that all the columnar cells cannot be transformed into flat cells. What becomes of those that are not so transformed? A partial answer to this question is suggested by the very characteristic appearance of the anterior pole in the older swimming larvae (Fig. 30). As may be seen in this figure, the nuclei of the ectoderm cells are arranged in a dense zone, except at the anterior end, where they are much less densely packed, and where they form a columella-like mass projecting some distance into the interior of the larva. The cells composing this mass are so small that I cannot speak of their outlines with certainty, but they appear to be spindle-shaped. The mesoderm cells at this end of the larva are nearly all spindle-shaped, as may be seen in the figure, and the general appearance of the region suggests that the ectoderm cells are migrating at this pole into the interior of the larva. With my small store of facts this must remain a mere conjecture, and yet the point seems worth mentioning.

After the larva reaches the stage shown in Fig. 27, it sinks to the bottom and attaches in the following manner. Keeping its spicular pole applied to the bottom of the dish and its long axis more or less vertical, it begins to rotate. The rotation lasts for several hours, and may be interrupted by the larva moving to a new quarter of the dish, there to begin again its monotonous rotation. All this time the transformation of the ectoderm is taking place. After the ciliated ectoderm has

become confined to the anterior (or upper in rotation) pole, the larva ceases to rotate and applies itself to the dish obliquely, that is in the plane $x-y$ of Fig. 27. It then flattens out at its spicular pole, and in this stage is shown in Fig. 28. The flattening out continues and the patch of columnar ectoderm grows smaller, until the young sponge has assumed a flat cake-like shape. In this condition it is approximately circular in outline (see Pl. XVIII, Fig. 55, surface view of recently attached sponge), and is entirely covered with a flat epithelium, and is practically solid. The straight spicules, which in the swimming larva formed a loose bundle at the posterior end, become distributed during the flattening of the sponge, through all quarters of the body. After the flattening is completed, as is shown in Fig. 55, the spicules project slightly all over the upper surface. The outline of the sponge soon becomes irregular, and the body undergoes many changes of shape, which, however, are so slow and gradual as to escape notice, unless drawings of the outline are made at intervals. In the solid body of the sponge the canals and flagellated chambers appear as separate cavities, which subsequently unite with one another; and the pores and oscula make their appearance as simple perforations of the outer skin. All the essential features of the sponge body are established two or three days after attachment. At this time the area of the body is considerably greater than that of the swimming larva, but its actual bulk cannot much exceed that of the latter. After this stage, practically no growth occurred in the sponges I kept. They lived for weeks, but whether from lack of proper food or for some other cause, they did not continue to develop.

It sometimes happens that a larva attaches to the surface film of the water. In this case fixation takes place at the non-spicular pole, which flattens out to form a wide surface of attachment (see Pl. XVII, Fig. 37, vertical section through a larva so attached). The columnar ectoderm in such larvae metamorphoses in a different fashion from that ordinarily followed, in that the ectoderm over the surface of attachment becomes flat, while that on the sides is still columnar, as may be seen in Fig. 37. Of the larvae that attached in this way,

those I watched did not develop any further than the stage shown in Fig. 37.

In his memoir on *Spongilla*, Götte (6) claimed that the entire ectoderm of the larva was lost, the inner mass of cells giving rise to all the layers of the adult. This account was opposed to the earlier one of Ganin (7) who described the larval ectoderm as retained and becoming the ectoderm of the adult. In his preliminary paper on the development of *Spongilla*, Maas (15) stated that the larval ectoderm was not thrown off, but after loss of cilia and gradual flattening became the thin membrane-like ectoderm of the adult; and the excellent series of figures given in his later paper (14) retrace the process step by step. Some of the older writers, Metschnikoff (11) and Schmidt (22) described a partial or complete loss of the larval ectoderm in several silicious sponges during the metamorphosis; Barrois (1) believed that in his *Desmacidon* and *Isodyctia* larvae, the ectoderm was partially lost; and among the more recent investigators, Marshall (18) describes a partial loss of the ectoderm in *Reniera filigrana*. On the other hand, the flattening of the larval ectoderm and its transformation into the adult covering, has been observed not only in the case of *Spongilla*, but in other carefully studied silicious sponges: in *Chalinula*, Keller (10), and *Myxilla*, Vosmaer (34). For the views of Yves Delage and Maas on the relation of the larval ectoderm to that of the adult in *Esperia*, reference may be made to pp. 317-319. It seems to me that the alleged cases of total or partial loss of the larval ectoderm (ectodermic hernia) so completely lack the requisite detailed proof, that none of them can be accepted. In all such cases it is probable that the ectoderm is not lost, but is flattened into an extremely thin membrane.

Ectoderm. — In the flat epithelium into which the columnar ectoderm changes, the separate cells are at first easily made out (see Pl. XVII, Fig. 36, longitudinal section of a larva like that shown in Fig. 28). When the metamorphosis is complete, however (see Pl. XVII, Fig. 38, entire vertical section through recently attached sponge, and Pl. XVII, Fig. 44, ditto through an older sponge), the ectoderm on both upper and lower surfaces forms a very thin membrane, in which nuclei are discernible

here and there, but in which I could not make out the cell boundaries. The ectoderm covering the surface of attachment is noticeable for the deeply-stained thickenings found in it in comparative abundance (*pr. th.* in Fig. 38, and in Fig. 39, part of vertical section through recently attached sponge). These thickenings are shaped and distributed as if they might be nuclei, surrounded by an accumulation of protoplasm, but the stain reveals nothing but a homogeneous mass. These bodies are found, often of large size (Fig. 44), in the ectodermal membrane surrounding the sponge, of which I shall speak presently. In both situations they give the impression of degeneration products.

The little sponge, when the flattening out is completed, has a smooth and nearly circular outline, the mes-entoderm extending quite to the edge of the body. Pl. XVIII, Fig. 55, shows a surface view of such a stage. The ectoderm at the edge of the sponge soon begins, however, to grow out in the shape of a thin membrane which completely surrounds the sponge, extending outwards to a considerable distance. This membrane, *ec. m.*, is shown in Pl. XVII, Figs. 38, 39, 44 (vertical sections), and in Pl. XVIII, Figs. 56, 58 (surface views). Nuclei can be made out here and there in it, but the cell outlines are indistinguishable. Before continuing the description of this membrane, it will be necessary to say a word or two in regard to the mes-entoderm of the recently attached sponge.

The first change which the mes-entoderm of the larva undergoes during metamorphosis may be gathered from a comparison of Pl. XVI, Figs. 29, 30, and Pl. XVII, Figs. 36, 37. It will be seen that the formative cells increase greatly in numbers, and become distributed uniformly through the body. The pale, densely packed polygonal cells which occupy the posterior end of the swimming larva, gradually disappear, probably becoming transformed into the more independent and consequently rounded formative cells (see periphery of Fig. 36). The slender spindle cells which occupy the anterior end of the swimming larva, are in their turn distributed through all parts of the body. The next change in the development of the mes-entoderm can best be studied in surface views (see Pl. XVIII, Figs. 55,

56, 57). After the sponge takes on the round cake-like shape, the mes-entoderm becomes divided into two regions (Fig. 55), a main central region in which the formative cells are more or less rounded and pretty densely packed, and a peripheral zone in which the formative cells become branched and amoeboid, and in which they are loosely packed. The distinction between the two regions is conspicuous in Fig. 55, drawn under a low power; and the manner in which the peripheral zone is formed is clearly seen in Fig. 56 (surface view of a part of the peripheral region of a young sponge). Further development bestows on the peripheral zone the character of an exquisite intercellular network (see *p. s.*, Fig. 57, surface view of a small part of the peripheral region of a young sponge). In the sponge represented in Fig. 57, the cells of the network are of about the same size as the formative cells in the rest of the body; but as the sponge grows older, the cells of the peripheral zone grow smaller, many of them becoming delicate spindle-shaped cells. The peripheral zone after it has assumed this character, is shown more or less well in all the sections of older sponges figured (see Pl. XVII, Fig. 44, and especially Pl. XVIII, Fig. 49, the peripheral part of a section such as Fig. 44). The processes of many of the cells run directly into the ectodermal membrane, and strongly suggest an intercellular connection between the ectoderm and mesoderm in this region.

When the ectodermal membrane grows out round the periphery of the sponge, the peripheral zone of mes-entodermic cells, which is already differentiated from the central mass, begins to push out lobes and processes within the membrane which is at its inner edge obviously composed of two layers (Fig. 49). This brings it about that the inner mass of cells loses the smooth contour of younger stages (Fig. 55), its edge becoming, instead, jagged and irregular (Fig. 58). The changes of shape which the sponge undergoes at this time are due to the fluctuations of the edge of the mes-entoderm mass, not to amoeboid movements of the ectoderm cells. Sponges are sometimes found in which the mes-entoderm has pushed out processes of very considerable length between the layers of the membrane. Such a sponge is shown in Pl. XVIII, Fig. 54.

The two mesodermic processes, *m. p.*, do not come to an abrupt end, but die out imperceptibly. The ectodermal membrane, from the very beginning of its appearance, becomes covered with *débris*, much of which is organic. In Fig. 54, round the main body of the sponge are strewn little masses of degenerating cells, dead protozoa, and homogeneous, rounded masses which betray their organic nature only by their affinity for the stain, and which are evidently degeneration products. A good part of this *débris* looks as if it came from the sponge itself, as if it were composed of cells which had lost their connection with the sponge in some way, and then degenerated. Before completing the description of the ectodermal membrane, Pl. XVIII, Fig. 57, needs a word of explanation. The sponge was surrounded by an ectodermal membrane of average width, which in the region drawn was thrown, perhaps artificially, into a fold, *x*, close to the edge of the mes-entoderm.

My description of the peripheral region of the young sponge differs from that given by Maas for *Spongilla* (14), which he finds can also be applied to the case of *Esperia* (16). In the accuracy of my own observations as far as they go, I am confident; and those of Maas seem to have been made with such care that I am inclined to believe farther study will reconcile the two descriptions.

In the young *Spongilla*, Maas observed that the whole periphery becomes amoeboid. The formation of processes was followed in the living sponge, and it could be seen that a hyaline prolongation was thrown out far beyond the inner tissues, into which the inner cells slowly flowed, the outline then becoming more even. To this outer region, which is in constant motion, Maas gives the name of "der amoeboid Hof." The amoeboid "Hof" forms the peripheral zone of the sponge, and is composed of ectoderm. Internal to it is the body of the sponge, *i.e.*, the mass of mes-entoderm. The movements of the amoeboid "Hof" are due to the amoeboid movements of its constituent (ectoderm) cells. Silver nitrate preparations show that the cells at the extreme edge of the Hof are in active motion, throwing out pseudopodia, and combining to form lobes (the hyaline prolongations, the formation

of which was observed in the living sponge). It is possible that the ectodermal amoeboid "Hof" of Maas corresponds to my "ectodermal membrane," but I have never observed the peripheral cells to be amoeboid. Even if they were amoeboid, their movements could exert no influence on the shape of the mes-entodermic mass, merely for the reason that the edge of the ectodermal membrane is too far away from this mass. Maas does not describe the peripheral zone of amoeboid mes-entodermic elements, which is so conspicuous in the sponge I studied. The extensive ectodermal membrane I have described, which surrounds the body of the sponge, is perhaps confined to the silicious sponges, and may not, of course, be universal in them. I regard it as an excessive development of a simple layer of amoeboid ectoderm, such as clothes the attaching *Sycandra*, Schulze (25).

NOTE. — I find that owing to the extreme awkwardness of the term mes-entoderm, I have frequently used mesoderm as synonymous with it. No confusion will arise from this, if it is remembered that until the canal system is formed, the body of the sponge consists solely of two layers, — an outer covering (ectoderm) and an inner mass of cells (parenchyma, mes-entoderm, or mesoderm). After the canals are formed the term mesoderm is applied exclusively to the tissues lying between the ectoderm and the canal system.

Subdermal Cavities and Canals. — Both the subdermal cavities and canals arise as intercellular spaces in exactly the same manner. Intercellular spaces appear in the larva while it is attaching, *in. sp.*, Pl. XVII, Fig. 36. There are not many of them, and they are small and round. After attachment, Pl. XVII, Fig. 38, extensive cavities appear in the body, which are entirely independent of one another. The cavities formed directly beneath the upper surface are especially large, though shallow. These, *s. d. c.*, Fig. 38, are the subdermal cavities; the deeper lying spaces, *can.*, are the canals. At this time the mass of cells lying inside the ectoderm, the mes-entoderm, is, as has been said, largely composed of formative cells, with smaller slender cells scattered about here and there. The cells of the mes-entoderm are all connected together by delicate processes, and there are many indications that the ectoderm

cells too take part in this intercellular network. In Pl. XVII, Figs. 39 and 42, are shown parts of vertical sections through two sponges in the same stage as Fig. 38. Owing to the quantity of water in the sponge at this age, the tissues are extremely delicate and gelatinous, and the intercellular network in the best preparations is naturally more or less broken, with many of the cells fallen out of their proper places. That the canals and subdermal cavities arise as great intercellular spaces or lacunae in the mes-entoderm, can easily be seen in these figures. The lacunae when first established have no definite walls, but are merely surrounded by ordinary undifferentiated mes-entoderm cells, *can.*, Fig. 39. The cells immediately surrounding the cavity then begin to flatten, throwing out lateral processes in such a way as to form a more or less complete wall, in which, however, the component cells are of very irregular and diverse shapes, Fig. 42, and *can.*, Pl. XVIII, Fig. 47 (small part of a section, such as Pl. XVII, Fig. 44). The lining cells continue to flatten, ultimately forming a continuous investment of epithelioid cells, so thin indeed that they constitute nothing more than a nucleated membrane, *can. w.*, Fig. 47. In Fig. 44 there is shown a small canal, *can.*!, in which a part of the wall has reached the condition of a nucleated membrane, while the other part is still composed of cells which have not yet flattened out to any great degree. Cavities are developed everywhere directly beneath the upper surface, and there constitute, as has been said, the system of subdermal cavities, Pl. XVII, Figs. 38, 44, and 50; Pl. XVIII, Figs. 48, 51, 52, and 53. The spaces formed deeper in the tissue of the sponge become the canals. The number of subdermal cavities and canals is at first relatively small, so that the space occupied by the mesoderm is comparatively great, Pl. XVII, Figs. 38 and 44. But as new canals are formed, and as the cavities and canals gradually connect with one another, the mesoderm becomes reduced in quantity, and before long assumes the adult condition, in which it consists of uniformly thin trabeculae separating the various canals. The increase in the extent of the series of cavities may be seen in a comparison of Pl. XVII, Fig. 44, with sections through older sponges, Pls. XVII and XVIII,

Figs. 48, 50, and 51. In the latter two figures the adult condition of the mesoderm has practically been reached (compare section of adult, Pl. XIV, Fig. 2). Communication between the various cavities is established by simple perforation of the intervening tissue, the cavities in question growing towards one another, and finally meeting. In Pl. XVII, Fig. 45, it would seem that the two canals, *can.'* and *can''*, have but lately met; and in Fig. 44 the canal, *can''*, has made connection with the subdermal cavity, *s. d. c.*

A more comprehensive idea of the formation of subdermal cavities and canals may be obtained from a study of surface views. In Pl. XVIII, Fig. 55, the earliest cavities are shown, as yet surrounded only by undifferentiated mes-entoderm cells. Two cavities in the same early stage of development are likewise shown in Fig. 56. In the sponge drawn in Pl. XVIII, Fig. 58, in about the same stage as Pl. XVII, Fig. 44, the cavities are numerous and a higher power would show they were lined by an epithelioid membrane. The cavities shown in this figure, as those in Pl. XVIII, Fig. 54, all lie directly beneath the surface. Other deeper lying cavities are present, but these naturally are not obvious. The smooth rounded outlines of the cavities coupled with the extreme transparency of the overlying sponge tissue (dermal membrane) at first sight makes many of the cavities appear as oscula (Figs. 54 and 58), but examination soon reveals the membrane covering them.

Dermal Membrane.—The portion of the sponge body which directly covers the subdermal cavities, develops into what is known as the dermal membrane. In its adult condition, *d. mem.*, Pl. XIV, Fig. 2, and Pl. XVIII, Figs. 48, etc., it consists of three layers: on the outside the ectoderm, on the inside the epithelioid lining of the subdermal cavities, and between the two a layer of mesoderm consisting for the most part of slender spindle-shaped or fibre-like cells (comp. Pl. XIV, Fig. 4). The first stages in the formation of the membrane are shown in Pl. XVII, Figs. 38, 39, 42. As the lining cells of the cavities flatten out, the superjacent mesoderm cells grow smaller and become transformed into spindle-shaped or branched cells, most of which lie in planes parallel to the surface. In the somewhat older stages,

Pl. XVII, Figs. 44 and 45, the mesoderm cells of the dermal membrane still retain some trace of their former plump protoplasmic body, but in stages yet older, Pl. XVIII, Figs. 48, 51, the cell body consists of a mere covering for the nucleus, continued into, in the majority of cases, two long slender processes. The large number of such fibre-like cells converts the dermal membrane of the oldest stages reared into a tough, strong covering. In surface views the dermal membrane can best be studied over the subdermal cavities, where the slender bipolar cells of the middle layer of the membrane are very conspicuous (Pl. XVIII, Figs. 58 and 59, the latter representing a part of the peripheral region of a sponge like Fig. 58). The basal portion of the sponge undergoes a development somewhat similar to that of the upper crust. Many of the mesoderm cells in this region become transformed into bipolar or branched cells with very small bodies and slender long processes; compare the successive stages shown in Pl. XVII, Figs. 42, 44 and 50. Scattered amongst the bipolar cells are quite a number of larger rounded or branched (formative) cells, Pl. XVII, Fig. 50 and Pl. XVIII, Fig. 51. In this part of the sponge flagellated chambers are not developed. To be sure while the canals are still few and wide apart, a few flagellated chambers may be found close to the basal ectoderm, Pl. XVII, Fig. 44, but after the system of canals becomes more extensive the basal portion of the sponge is no longer found to contain any chambers. The same is true of the dermal membrane, in which during the earlier stages there is occasionally (very rarely) found a chamber, Fig. 44, but which in later life is entirely devoid of such structures.

Efferent Canals and Oscula. — Efferent canals are formed in this way. Some canal which usually extends deep into the tissue of the sponge (*cf. c.*, in Pl. XVII, Fig. 50, is very probably going to develop into an efferent canal) breaks through to the exterior by a large opening, the canal becoming the efferent canal, the opening the osculum, Fig. 57. The osculum is produced by simple perforation of the dermal membrane, the ectoderm becoming continuous round the edge of the aperture with the lining of the canal. Oscula may be formed anywhere on the surface of the sponge, in the central region of the upper

surface, *os.*, Pl. XVIII, Figs. 48, 51, 52, at the extreme periphery, Pl. XVII, Fig. 45, and Pl. XVIII, Fig. 52, and even on the under surface, Pl. XVIII, Figs. 52, 53. The appearance of oscula on the under surface is not of frequent occurrence, still I have found several in this position. The number and distribution of the oscula in these young sponges, as in the adult, is quite without regularity. There may be one or several, and they may be anywhere on the surface of the sponge.

Afferent Canals and Pores.—The subdermal cavities in the young sponges act directly as afferent canals. The membrane above a cavity becomes perforated by pores, and in the floor develop flagellated chambers, Pl. XVII, Fig. 50, and Pl. XVIII, Fig. 51. In Fig. 51, for instance, it is plain that the water entering into the subdermal cavity, *s. d. c.*, must pass directly through the flagellated chambers in order to get into the efferent canal, *ef. c.* In the adult, many of the subdermal cavities have a floor made up of a layer of flagellated chambers (*s. d. c'*, Pl. XIV, Fig. 2), and in such cases it would look as if the water passed directly from the cavity into the chambers. Though the subdermal cavities undoubtedly act directly as afferent canals in the young sponge, other afferent canals are also developed. Some of the deeper lying cavities establish connection with the subdermal spaces, for instance *can.*" in Pl. XVII, Fig. 44, and these, it would seem, become afferent canals.

In the sponges I reared, the number of pores that developed was not very great. In the oldest individuals each subdermal cavity had as a rule one or a few pores, Pl. XVIII, Figs. 58 and 59. The pores developed as perforations, the edge being strengthened, as is true also of the edge of the oscula, by the presence of fibre-like mesoderm cells. In many cases a very fair number of pores had developed before an osculum made its appearance. This is true of the sponge shown in Fig. 58. The distribution of the pores in the young sponge may be gathered without further description from the two Figs. 58 and 59. A curious phenomenon analogous to the formation of a pore sometimes takes place in the peripheral zone (*p. z.*, Fig. 57), an instance of which is shown in Fig. 59, *p. for.* The peripheral zone is perforated from one surface to the other by an aper-

ture of about the size and of the same character as a pore. Such peripheral foramina (*p. for.*) are not common in *Esperella*, and seem to have no function.

Flagellated Chambers.—After the larva attaches, Pl. XVII, Figs. 36, 37, 38, it is, as has been said, largely composed of formative cells. Other smaller cells, bipolar or otherwise branched, are scattered amongst them. Moreover, all the mesentoderm cells are united into a network, Pl. XVII, Figs. 39, 42. The formative cells, many of them at any rate, are multinucleate. In Pl. XVII, Fig. 46, a group of formative cells is shown, some of which are multinucleate. These possess, besides the central larger nucleus with its nuclear membrane and chromatin mass, one or more smaller peripheral nuclei, each having its chromatin mass with nucleoplasm and surrounding membrane. The peripheral nuclei at first sight look like mere chromatin spots, but more careful study satisfied me they were surrounded by nucleoplasm and a membrane. There are, however, scattered about in the cell protoplasm other bodies which stain like chromatin balls, but which are in all probability yolk granules. Two of these are shown in the lowermost cell of Pl. XVII, Fig. 46.

In *Spongilla*, Götte (6) has described multinucleate cells, which break up by a process analogous to budding, and form cell-groups which give rise to the flagellated chambers. The multinucleate cells are derived from mesoderm cells containing a nucleus and large yolk granules, the yolk granules becoming transformed into nuclei! Maas (14) has studied the same cells ("Dotterzellen") in *Spongilla*, using a differential stain (Lyons blue and carmine, or malachite green and carmine). He thinks that the cells in question contain only a single nucleus, together with a number of yolk granules of varying size. The nucleus stains red, the yolk granules blue. Maas does not believe that these cells are concerned in the formation of the chambers, but describes the latter arising as diverticula from a main entodermic cavity. Maas has also studied (16) what I have called "formative cells" in *Esperia*, and does not believe they are multinucleate. The bodies which I regard as small nuclei peripherally placed, he thinks

are not nuclei, but are products of cell metabolism. He further believes that these cells (formative cells) do not give rise to the flagellated chambers. For a statement of his views on this head, see p. 319.

Now, the precise way in which the flagellated chambers are formed in *Esperella* depends on the behavior of the formative cells. The simplest way in which a chamber is ever formed is for several formative cells to group themselves together in a hollow sphere, *f. c.¹* and *f. c.²* in Pl. XVII, Fig. 42, and *f. c.* in Pl. XVII, Fig. 39. They then divide up into smaller cells, which gradually acquire the characteristic features of collared cells. In the chamber *f. c.²*, Fig. 42, the division into smaller cells has already progressed to some extent, but the cells still retain their rounded independent shape, one of them remaining much larger than the others. In the surface view, Pl. XVIII, Fig. 57, a number of chambers are shown which, I take it, are being formed in the manner described. In *f. c.¹*, *f. c.²*, *f. c.³* the formative cells are as yet only loosely combined, especially loosely in *f. c.¹*. In *f. c.²* the connection between the separate cells is a closer one, and some of the cells have divided, as is evidenced by the difference in size. In most of the other chambers, of which *f. c.⁴* may be taken as an example, the division of the cells has been carried so far that they are tightly compressed and more or less columnar. Early in the development of the chamber the nuclei acquire the characteristic appearance of the nuclei of collared cells, becoming small, and staining very deeply. The development of the collar I was not able to follow. As formed in this way, a flagellated chamber is nothing more than an intercellular space or lacuna, and in its first stages is essentially similar to a canal (compare in Fig. 42 the canal *can.¹* and the chambers *f. c.¹* and *f. c.²*), the lacuna becoming the cavity of the canal or chamber respectively. This means of producing flagellated chambers is only employed in the early stages, directly after fixation. In a few sponges, at this time, it seems almost the only means employed, but in most individuals it is made use of side by side with another method, which I shall now describe.

The formative cells in the recently attached sponge tend to break up into solid masses of much smaller cells. Such masses are common, several of them being shown in Fig. 39 and one in Fig. 42 (*c. m.*). The multinucleate condition of so many of the formative cells must be regarded as preliminary to division, the division resulting in some cases in the production of the masses just spoken of. In other cases, where the formative cells, as such, have grouped themselves in the shape of a chamber, cell division merely leads to increase in the number of the enclosing cells. In other cases again, no doubt, the formative cells break up into finer cells, which separate and become scattered about. In whichever way they are used up, the number of formative cells, which is very large in the just attached sponge, grows steadily smaller during the production of the canals and chambers. The solid masses of small cells to which certain formative cells give rise, are irregular in shape, and retain their connection with the cell network, Pl. XVII, Figs. 39, 40, and 41. The small cells of which such solid masses are composed quickly acquire the characteristic nucleus of the collared cell, and the mass itself constitutes the anlage of a flagellated chamber. I do not, however, believe that a single formative cell, unaided, gives rise to a flagellated chamber. On the contrary, I believe that several of the smaller solid masses of cells, each of which has been derived from a single formative cell, unite to form one of the larger masses, and this develops into a flagellated chamber. In looking over Figs. 39, 40, and 41 it is seen that the masses of cells are of various sizes; and while it is permissible to assume that one of the smaller masses can reach by simple growth to the size of one of the larger masses, the close connection which exists between many of the smaller masses (*c. m.* in Fig. 39), coupled with the shape of some of the larger masses (*an. f. c.* in Fig. 39; this mass has already acquired its cavity), creates the impression that the latter have been formed by the fusion of the former. The solid mass of cells so formed acquires a central cavity, which at first is extremely small, *an. f. c.*, Figs. 40 and 41. While the cavity is quite small, the surrounding cells are packed in several layers, but as the cavity increases

in size they become arranged in a single layer, *an. f. c.*'', Fig. 40. The surface of the flagellated chamber so formed gradually becomes smooth, and its shape, which may be of almost any character (*an. f. c.*'', *an. f. c.*'', Fig. 41, and *an. f. c.*, Fig. 42), becomes spheroidal.

The two methods of forming the flagellated chambers just described are distinct methods, though, as will be pointed out, one may be regarded as a modification of the other. That the two methods are distinct, that one is not a mere stage of the other, must be evident from the description. On the one hand we have solid masses of quite small cells, of a characteristic appearance, giving rise to a chamber; on the other, formative cells are found grouped in a hollow sphere (only in rare instances do these large cells form solid groups, *for. c. g.*, Pl. XVIII, Fig. 57), giving rise directly to a chamber. After the system of cavities has got well started, Pl. XVII, Fig. 44, and Pl. XVIII, additional chambers are formed, I think, exclusively after the second method; at least no hollow groups of formative cells are found, but, on the other hand, solid masses of small cells are comparatively abundant.

In some few individuals the chambers are formed in yet another fashion. The cells of the just attached sponge may nearly all split up into fine cells, so that the mes-entoderm is transformed into a nearly uniform mass of fine cells, with a few larger (formative) cells scattered about here and there. In Pl. XVII, Fig. 43, is shown a part of a vertical section through such a sponge. In it one flagellated chamber, *f. c.*, is marked out. In a sponge which happens to develop in this way it seems that the flagellated chambers must be produced simply by the appearance of cavities or lacunae in the tissue, round which the cells arrange themselves in a regular wall. This manner of forming the flagellated chambers is obviously only an extension of the second method, in that very many of the formative cells early break up into masses of fine cells.

In the larva during the course of attachment one or two flagellated chambers sometimes make their appearance, as in Pl. XVII, Fig. 36, *f. c.*; but the details of the formation of such chambers were not worked out.

The three methods employed for the production of flagellated chambers in the recently attached sponge may be regarded as fundamentally the same, the second and third being modifications of the first. The third method I have already reduced to the second. The second method comes into existence because of the precocious division of the formative cells. A group of formative cells, instead of first arranging themselves round a central space and then dividing, divide and the masses resulting from their division become aggregated together and subsequently acquire a central cavity. Of the three methods the first is probably the most primitive, the other two having been derived from it. Viewed in this light, the flagellated chamber is formed in a manner essentially identical to that in which a canal is produced, and is, like the canal in its origin, an intercellular space. As may be seen in a later section of this paper, I am forced to believe that the formation of chambers and canals in the young *Esperella* as intercellular spaces, is best regarded as an instance of coenogeny.

The chambers increase in number with the increase in extent of the canal system ; and the number of formative cells and indeed the quantity of mesoderm in general, decreases at a corresponding rate. The gradual manner in which the distribution of the chambers, characteristic of the adult, is acquired, may be gathered from a comparison of the successive stages shown in Pls. XVII and XVIII, Figs. 44, 48, 50. In the later stages reared, the chambers are found to open into the canals, as is shown in Pl. XVII, Fig. 50, and Pl. XVIII, Fig. 48.

Spicules. — The long spicules found at the posterior end of the swimming larva become distributed through the body of the sponge during the course of attachment, Pl. XVII, Fig. 36. In the young sponge, after attachment, they are found with their sharp ends projecting for a short distance all over the upper surface, Pl. XVIII, Figs. 55, 58. Sections show that the projecting spicules do not perforate the ectoderm, but that they lift the ectoderm up, supporting it like so many tent poles, Pl. XVII, Figs. 43, 45. The first indications of the spicular bundles which support the dermal membrane in the adult, are to be seen in such stages as Fig. 44, where a few spicules are

shown lying near one another in the pillars of tissue separating the subdermal cavities. Other long straight spicules are scattered freely about in the deeper parts of the body. The bow-shaped spicules present in the swimming larva are found in small numbers distributed irregularly through the mesoderm of the attached sponge, Fig. 44. The embryonic shovels which in the swimming larva are united in rosettes, Pl. XVI, Fig. 30, and Pl. XVII, Fig. 34, are always found free in the attached larva. In the young sponges there are not many of these spicules, and the few to be seen are usually found in the dermal membrane, Pl. XVII, Figs. 44, 50.

Summary of the Leading Facts in the Gemmule Development of Esperella.

1. Gemmules appear in any part of the sponge mesoderm, and when present in large numbers, cause degeneration in the sponge tissue.

2. A number of mesoderm cells well supplied with yolk collect together and the mass so formed rounds itself off into a gemmule, the outer cells becoming the follicle.

3. The gemmule grows not only by cell division, but by the fusion with it of other small gemmules. It becomes a large mass of closely packed cells, full of fine yolk.

4. The gemmule, when mature, breaks up into irregular masses of cells, and these separate into the constituent individual cells.

5. The outer cells become ectoderm. Those at the posterior pole flatten, and develop neither flagella nor pigment. The other ectoderm cells become columnar, and develop both flagella and pigment.

6. The inner mass of cells forms an intercellular network. It is a parenchyma in which there is no distinction between an ectoderm and a mesoderm. The parenchyma cells at the posterior pole become closely compressed.

7. In the swimming larva there is a bundle of long straight spicules in the posterior end. Bow-shaped spicules and embryonic shovels (in rosettes) are scattered through the parenchyma.

8. The ectoderm begins to flatten from the posterior pole forward during swimming life.

9. The swimming larva attaches itself by the posterior pole, but obliquely, so that it lies on its side.

10. During attachment the entire ectoderm grows flat, and afterwards spreads out round the sponge, as a membrane containing no mesoderm.

11. A peripheral mesodermic zone is formed, consisting of a network of cells. To the fluctuations in the edge of this zone are due the changes in contour of the young sponge.

12. The canals and subdermal spaces arise as lacunae or intercellular spaces in the parenchyma, which are at first independent of one another, and only subsequently become connected by the perforation of the intervening tissue. The parenchyma cells immediately surrounding the lacuna develop into a lining membrane of epithelioid cells. In their origin and method of formation, there is no difference between subdermal cavities, afferent canals, and efferent canals.

13. Pores and oscula arise in the same way, as perforations of the dermal membrane overlying the subdermal cavities and efferent canals respectively.

14. Flagellated chambers arise independently of each other and of the canals, only later acquiring connection with the canal system. A chamber may be formed from a group of formative cells which arrange themselves in a hollow sphere, the intercellular space becoming the cavity of the chamber. Or else the chamber may be produced by the appearance of a central cavity in a solid mass of fine cells, derived from the division of formative cells.

Previous Knowledge of the Development of Esperia (= Esperella). — Besides the older observations of Metschnikoff (11), Carter (2), and Oscar Schmidt (22) — see p. 370, section VI — on the development of this genus, there are papers dealing with the subject by Yves Delage (36, 1890), Maas (16, 1892), and myself (35, 1891).

Yves Delage describes an incomplete layer of rounded cells at the surface of the larva, between which protrude the peripheral ends of the ciliated cells. The ciliated cells migrate into the interior of the larva, subsequently forming the lining epithelium of the canals, and are regarded by the author as constituting the endoderm. The superficial rounded cells then form a continuous layer and constitute the ectoderm. These facts enable the author to make a detailed comparison between the amphiblastula of calcareous sponges, and the solid larva of the silicious sponges. The former is hollow, but the cavity of the latter is filled with a mass of mesoderm (precociously formed, as compared with the amphiblastula development). And instead of the endodermic and ectodermic elements being confined to opposite halves of the larva, as in the amphiblastula, they are, in the solid larva, intermingled over the whole surface. Consequently, in the latter, the endoderm (ciliated cells) cannot invaginate as a continuous layer, but the component cells have to migrate into the interior separately. The flagellated chambers are formed from special mesoderm cells.

The description of the larvae of *Esperella* Lorenzi and *E. lingua*, given by Maas, agrees in essential points with my account of the larva of *Esperella fibrex*. The resemblance holds good even for many details. On the other hand, Maas describes a cavity or lacuna in the anterior end of the larva, traversed by branching cells, of which I saw no sign in the form I studied. Again, the chelae, which, in Maas's larva as in mine, are united in spherical groups, are in the former differentiated shovels, while in the latter they remain in a more embryonic condition. A more important difference is exhibited in a layer of cells which Maas describes at the anterior end of the larva, with nuclei very close to the periphery, and which he believes to be "intermediary cells," *i.e.*, cells lying between the ectoderm elements proper. I have frequently seen nuclei here and there very close to the periphery, but saw no reason for regarding them as belonging to a set of cells distinct from the ectoderm. Maas suggests that the arrangement of the spicules in the swimming larva is such that the weight is evenly distributed round the long axis—an

arrangement evidently adapted to the rotating swimming habit of the larva. This seems to me an excellent suggestion, and is very probably the true explanation of the arrangement of the spicules in such larvae.

Maas's views on the relation of the layers of the sponge body after metamorphosis to the layers of the larva may be read in the following extract (*l.c.* p. 426): "Die festgeheftete Larva besteht auf diesem Stadium wie die freischwärmende hauptsächlich aus zwei verschiedenen Gewebsschichten, die im Aussehen ganz dieselben wie die der Larve sind; es bleibt daher nichts anderes übrig als anzunehmen, dass die innern und untern Zellen mit kleinern Kernen eines solchen Stadiums, dieselben sind, wie die äussern kleinkernigen Elemente der Larve, und dass die obern und äussern Zellen des gerade angehefteten Stadiums den innern und hintern Zellen der Larven entsprechen, dass also beide Zellschichten in der Metamorphose (wie bei *Sycandra raphanus*) ihre Lage zu einander verändert haben. Dieser Wechsel scheint mit dem Ansetzen in directer Verbindung zu stehen, indem die Masse der kleinkernigen Elemente am Vorderpol zusammengedrängt wird, und indem zuerst am Hinterende, dann von allen Seiten die Zellen der innern Schicht um sie herum wachsen." As may be seen from this passage (see also Maas's recent paper in the *Biolog. Centralblatt*, 17, p. 570), the author's views on the morphology of the larva are fundamentally different from mine. He regards the larva as composed of two layers—a layer of ciliated columnar cells covering the body except at the posterior pole, and a layer of irregularly-shaped cells filling the interior and covering the surface at the posterior pole. (Now that I have shown that the covering cells of the posterior pole and the ciliated columnar cells belong to the same early layer, Maas's views on this point seem to me untenable.) Further, the author believes that during the metamorphosis the columnar ciliated cells migrate into the interior of the attached sponge, forming the flagellated chambers and, in part at least, the efferent canals; while the inner cells of the larva take up a position on the surface, forming the adult ectoderm and the rest of the sponge body. The author's views on this

point approach those of Delage. That the solid larva turns itself inside out in this fashion is certainly a remarkable phenomenon, and one that calls for abundant evidence. Maas's argument, as far as I can make it out, is that the attached sponge consists of two layers which exactly resemble those of the swimming larva, but that the inner cells in the attached sponge are like the outer cells of the larva; and, conversely, the outer cells of the attached sponge are like the inner cells of the larva. It seems to me that deductions made from histological similarities of this sort can never be relied on with much confidence. And especially must this be true in a case like the one in hand, where so many of the cells are undergoing histological change. I cannot see that either Delage or Maas proves his case. I have, as has been mentioned, found indications that some of the ectoderm cells of the larva migrate into the interior during metamorphosis, but I found no evidence that the ectoderm as a whole does not continue on the surface.

Maas finds that the subdermal spaces, canals, and chambers arise separately, the spaces and canals as large lacunae in the parenchyma of the sponge. Maas does not believe that the cells which I have called "formative cells" have any share in producing the flagellated chambers. He thinks the chambers are formed from aggregations of the small cells with small nuclei "which in the larva constituted the ciliated epithelium, and during the metamorphosis migrated into the interior" (16, p. 432). Maas believes that the efferent canals (in part, at least) are formed by similar cells having the same origin. I must confess that all this seems to me highly theoretical, the whole belief resting on a partial histological resemblance between the ciliated cells of the larva and the cells of which the finished or nearly finished chambers are made. As for the canals, I have always seen them formed by cells bearing no resemblance at all to the small slender cells which Maas supposes to be migrated ectoderm cells. In regard to the chambers, I am disposed to believe that the aggregates of small cells described by Maas are not different from those I have described as resulting from the division of larger cells.

II. ADULT STRUCTURE AND GEMMULE DEVELOPMENT OF TEDANIA BRUCEI, N. SP.

I. ADULT.

Diagnosis.—*Tedania Brucei*, n. sp. Sponge body large, usually massive, though sometimes forming flat incrustations. Color red, and body firm and fleshy. Surface marked with shallow meandering furrows and low intervening ridges, the tissue of the ridges being firm, that of the furrows gelatinous and containing large canals. The dermal membrane contains microscleres (oxeas), and is perforated by numerous large rounded pores, both over the furrows and ridges, opening into subdermal cavities. Oscula usually large and conspicuous and often at the end of oscular papillae. The main efferent canals surrounded by a large amount of gelatinous tissue; the body of the sponge is thereby broken up into an interlacing network of gelatinous and dense tracts. Flagellated chambers found only in the latter. Flagellated chambers open directly into afferent and efferent canals. The spicules include slightly curved strongyloxeas (Sollas) $\frac{2.0}{1.0}$ to $\frac{2.5}{1.0}$ mm. in length, tylotes with sharply nicked heads of same length, and very slender oxeas varying in length from $\frac{2.0}{1.0}$ mm. to microscleres of $\frac{4}{1.0}$ mm. and less. Skeleton of dense regions of sponge consists of a close and confused meshwork of strongyloxeas; a few bundles composed of tylotes and oxeas, crossing the meshwork; and brushes of tylotes supporting the dermal membrane, which in gelatinous regions give place to bundles of tylotes following curves of subdermal cavities. Gelatinous tracts contain scattered tylotes and oxeas, the latter from $\frac{2.0}{1.0}$ mm. to microscleres. Microscleres especially abundant round larger canals, often radially arranged. — *Green Turtle Cay, Bahamas.*

This very handsome sponge is abundant in the quiet waters of the "sounds" or deep bays which run into the island. It is especially found on the roots of the mangrove, which grows luxuriantly round the borders of the sounds, though it may also be found on the bottom, where it is apt to become incrusting. Its large size, often ten inches in diameter, and bright

red color make it exceedingly conspicuous. The specimens found on the mangroves are generally more or less ovoid in shape, with two or three oscular papillae, an inch or two long, projecting from the upper surface. The surface is furrowed in the most intricate and irregular fashion (Pl. XIX, Fig. 60, shows a small portion of the surface). The furrows are generally very shallow, but are conspicuous both in fresh and alcoholic specimens, because the tissue here is gelatinous. In the depigmented alcoholic specimens, the furrows look much darker than the ridges. The ridges, as will be seen in the figure, exhibit numerous rounded and slight elevations, which in places may appear as well-marked papillae. On the oscular papillae the furrows often become regularly arranged, when they pursue a comparatively straight course towards the end of the papilla, meeting one another at acute angles. At the end of each papilla there is usually a single osculum leading into a shallow cavity, into which open several large efferent canals. There may, however, be two or three oscula set close together on the end of the same papilla.

The division of the sponge body into the interlacing network of dense and gelatinous tracts mentioned above is shown in any section. Pl. XIX, Fig. 61, is a section vertical to the sponge surface, including two furrows, *f*, with the intervening ridges, *r*. Both the superficial gelatinous tracts directly beneath the furrows, as well as the deeper ones, show in their centre one or more large canals (efferent canals). The dense tracts alone have the true sponge tissue, the gelatinous tracts containing no flagellated chambers. The difference between the two is made the more striking in that the dense or spongy tracts contain a close meshwork of spicules, which is absent in the gelatinous tracts. Pl. XIX, Fig. 64, is a small portion of such a section as Fig. 61 with skeleton omitted, and shows a part of a superficial gelatinous tract, *g*, together with a part of the adjoining spongy tract, *sp*, in which the higher magnification permits the flagellated chambers to be shown. Pl. XIX, Fig. 62, is a section across the base of an oscular papilla with skeleton omitted, and shows the connection between a superficial gelatinous tract and a deep lying one. Like the network

of sponge tissue, the gelatinous tracts form a connected system in which run the main efferent canals.

The dermal membrane, Pl. XIX, Fig. 66, contains numerous microscleres (oxeas), and the supporting brushes of tyloles are usually torn away with it. The pores are thickly distributed over most of the surface, but there are aporous or nearly aporous tracts found here and there. The pores lead directly into subdermal cavities, *s. d. c.*, Figs. 61 and 64, which are in general smaller in the spongy regions than in the gelatinous (Fig. 61). Even in the spongy regions, Fig. 64, the subdermal cavities are surrounded by a certain amount of gelatinous tissue, there being very few flagellated chambers in their immediate neighborhood. The subdermal cavities, both those under the ridges and the furrows, communicate in an irregular fashion with one another and open into main afferent canals, *af. c.*, Fig. 64, which, it will be seen from Figs. 61 and 64, enter the dense mass of flagellated chambers directly from above, and from the superficial portions of the gelatinous tracts as well. The main afferent canals subdivide, their terminal divisions opening laterally into the flagellated chambers, as is shown in Pl. XIX, Fig. 65 (*af. c.*), this figure representing a small portion of the mesoderm of *Tedania*, showing flagellated chambers and both afferent and efferent canals. The flagellated chambers open in the same manner into the efferent canals. The water passes out of the spongy tracts by numerous efferent canals distributed along the margin of the spongy and gelatinous tracts. These canals are well shown round the edge of several of the gelatinous tracts of Fig. 62. They open into the one or more larger vessels (main efferent canals) lying in the central part of the gelatinous tracts (Figs. 61 and 64, *ef. c.*). The main efferent canals have a denser wall than the rest, which is usually well provided with microscleres, arranged in a radial fashion. These canals communicate with one another and open at the oscula, as described above. The communication of a superficial efferent canal with a deeper lying one is partly shown in Fig. 62, *com. ef. c.*, the plane of the section cutting the connecting canal into two portions. The superficial efferent canals are especially interesting in the upper part of

the oscular papillae, or in the upper part of young sponges of a conical shape, a few inches high. In both the oscular papilla and the upper part of the young sponge, the furrows or superficial gelatinous tracts run with comparative directness straight towards the upper end. An indistinct radial symmetry is thus given the papilla, which appears more pronounced after the efferent canals are studied. For it nearly always happens that the papilla has in its axis some one canal larger than the rest, and each superficial gelatinous tract has likewise, as a rule, one large efferent canal, so that near its upper end the efferent system of the papilla consists of a central canal, round which are disposed several superficial canals, all either opening by a single osculum or by two or three separate but closely adjoining oscula. Pl. XIX, Fig. 63, represents a section cut from a young sponge of a conical shape and some five inches high. At the apex of the sponge was a single osculum, and the section drawn was cut a short distance below it. In this region the superficial gelatinous tracts contain no subdermal cavities. All that each contains is a single efferent canal running parallel to the surface and towards the apex, *sup. cf. c.* in Fig. 63. Of these, three appear in the section. A fourth unites at this level with the central canal, *c. cf. c.* Lower down the number of superficial canals increases, but they no longer run in the same direction, pursuing, on the contrary, the irregular meandering course characteristic of the general surface. The canals shown in Fig. 63 all open by a common orifice, which I have spoken of as the osculum, at the apex of the sponge.

The flagellated chambers are spheroidal, and the mesoderm of the spongy tracts is comparatively abundant, consisting of rounded or branched amoeboid and spindle-shaped cells. The gelatinous tissue is composed of a network of cells with an abundance of watery jelly in the interstices. The cells as a rule have small bodies and several long slender processes, Fig. 64.

The tylotes found in *Tedania B.* all have nicked heads as in Pl. XX, Fig. 67 *c.* This seems to be a common variation in the species of *Tedania*. Oscar Schmidt in speaking of the Atlantic *Tedanias*, says: "Die meisten dieser *Tedanien* besitzen

neben den Doppelkeulchen mit glatten Köpfen solche, wo auf dem Scheitel des Kopfes einige kleine Knötchen entstehen. Diese letztere Form nimmt in einzelnen Individuen überhand, und ist in noch anderen ausschliesslich vorhanden." All the individuals of *Tedania* B. examined agreed in this respect, and it seems proper here to regard the nicked head as a specific characteristic. Tylotes are found free and scattered about in the gelatinous tissue. Round the subdermal cavities of this tissue they form loose bundles which follow the curves of the cavities, Pl. XIX, Fig. 61. The brushes of tylotes which support the dermal membrane have mixed with them a number of microscleres (oxeas). Crossing the meshwork of spicules and pursuing an entirely independent course, are found here and there a few long and slender skeletal bundles consisting largely of tylotes intermingled with oxeas. The oxea, Pl. XX, Fig. 67*b*, aside from the situations just spoken of, is found in abundance in the gelatinous tracts where it varies in size, as has been mentioned, from the dimensions figured to those of a microsclere, the latter form being especially common round the walls of the larger efferent canals. The skeletal meshwork is made up exclusively of strongyloxeas, which are all as is shown in Fig. 67*a*, slightly bent. I have spoken of this meshwork as confused. It is confused in the first place because the spicules are so closely packed, that the meshes are not bounded by single spicules but by little bundles. And in many places it so happens that the spicules are arranged in such a way that they both bound the meshes and help to form a continuous skeletal bundle. Two such bundles are shown in Fig. 61, which fairly well represents the meshwork of spicules.

The homology between pores and oscula upheld by Barrois (I) and others, receives perhaps some additional support from the occurrence in *Tedania* of such openings as those shown in Pl. XX, Fig. 68 (a small portion of the surface). These openings lie in the gelatinous furrows, and in their immediate neighborhood there are but few pores. They are larger than the pores, but very much smaller than the ordinary oscula, and may therefore be classified as structures intermediate between the two.

Collecting and Embryological Methods.—During August, September, and October, the *Tedania*s at Green Turtle Cay were found to contain large numbers of embryos which turned out to be gemmules, the development of which is essentially like those of *Esperella*. The embryos were imbedded in the mesoderm, but though they were abundant, they were not sufficiently so to cause any breaking down of the sponge tissue. The mesoderm of *Tedania* B. is a bright red and the gemmule embryos which were present in many stages were of the same color. If a *Tedania* is put in an aquarium, almost at once ciliated larvae begin to be cast out of the oscula. For the purpose of obtaining embryos, I found it was useless to keep the adult sponge more than two or three hours. During that time they throw out a good many embryos, but afterwards scarcely any. By changing the water frequently, large as the adults were, I could keep them alive for many hours, but after the first couple of hours they, like so many other marine animals, lose their irritability, and eject no more embryos. In order to get a great number of embryos it was therefore necessary to collect many adults, keeping each of them but a short while. In bringing a large sponge like *Tedania* B. from the collecting grounds to the laboratory, care should be taken to supply it with an abundance of water, and if it must be lifted out of the water, let the exposure to the air be as short as possible. It will be found well to support the sponge with one hand just below the surface of the water, and with the other dip a bucket beneath it. In this way a sponge may be brought into an aquarium without having been out of the water for a moment. It being a matter of considerable time and labor to bring so many sponges from the collecting grounds to the laboratory in a small sail boat, I tried on a few occasions getting my larvae directly on the grounds. Paddling along the mangroves at the head of "Black Sound," whenever we saw a good sponge, my negro boy or I would fish it up and carefully bring it into one of the two large tubs I kept full of water in the bottom of the boat. When we had pretty well filled one of the tubs, I would wait fifteen minutes and then transfer all the sponges to the other tub, and begin examining the water

of the first. This was done by dipping it out with a two-gallon glass aquarium jar, in which the bright red sponge larvae could easily be seen swimming about. These were sucked out with a pipette and put into an aquarium jar well protected from the sun. In observing and dipping at the larvae, the negro boy soon became expert, and proved himself of very considerable use for this purpose. By the time we had finished examining the water of the first tub, the water in the second would contain enough larvae to be worth looking through in this manner. The sponges were then thrown away and another lot collected.

Having obtained a stock of the swimming larvae (for several reasons I had need of a large number) they were then put in flat shallow dishes in which I wished them to attach. Many of these dishes I coated with paraffine, allowing the larvae to attach to the paraffine, as already described for *Esperella*. In other cases the larvae attached to the walls of the dish, or to cover-glasses placed on the bottom. As in the case of *Esperella*, the whole process of fixation could be observed with perfect ease by placing one of the small paraffine-coated dishes on the stage of the microscope, using reflected light and a low power. The larvae swim about for a day, as a rule, and then attach, undergoing a metamorphosis essentially like that of *Esperella*. The just attached sponge is a thin incrustation-like mass, in which the canals, flagellated chambers, *etc.*, appear in the course of a couple of days. The sponges I reared lived indefinitely in the aquaria, but did not increase in size after the first two or three days (and that increase was probably one of area alone, not of bulk), except in a single case where the little sponge, when killed, had reached a diameter of nearly a quarter of an inch, but had not gone beyond his brothers in morphological differentiation. I attempted to get older stages in a way which it certainly seems should have been successful, but which was not. Having allowed the larvae to attach to pieces of wood or glass, I tied these pieces to the mangrove roots, in the very home of the sponge, but even there the little sponges did not increase in size. The pieces of wood hung to the mangroves were in some cases protected by wire cages, and in

others not. The protection made no difference. The protected and unprotected sponges lived for two or three weeks (and no doubt would have lived much longer if I had not grown tired of the experiment), undergoing practically no change, and apparently exempt from attacks on the part of the little fish and crustacea which swarmed round the mangroves.

In preserving the *Tedania* material I was not as fortunate as I was later in the case of *Esperella*. For the *Tedania* embryos (larvae and attached stages also) I used Perenyi's fluid and also osmic acid. Neither is nearly so good as the Zacharias mixture already spoken of, though of course they give fairly good results.

2. DEVELOPMENT OF THE SWIMMING LARVA.

Formation of Gemmules.—The gemmules are not confined to any particular part of the body, but are distributed more or less uniformly through the mesoderm. The very young gemmules are simply imbedded in the mesoderm, Pl. XX, Fig. 69*g*., but the ripe gemmules, *r. g.*, Pl. XX, Fig. 71, are provided with a definite sheath, *g. sh.*, and are immediately surrounded by good sized canals. The gemmule of *Tedania* is puzzling, and I cannot claim to have actually disclosed its true structure. Still, the facts I have discovered, when compared with the development of *Esperella*, make it very probable that the gemmule of *Tedania* has essentially the same structure as that of *Esperella*.

The ripe or full-sized gemmule of *Tedania* is a large spheroidal mass, in which neither cell boundaries nor nuclei can be made out. It is densely and uniformly granular, the granules being fine yolk granules (like the yolk in the cells of the *Esperella* gemmule) which stain well with any stain I tried (haematoxylin, carmine, cochineal, and other aniline stains). Repeated attempts with many stains on the thinnest of sections have failed to reveal nuclei, but it is possible that the employment of a different killing fluid would lead to better results. Some idea of the puzzling appearance of the deeply staining, uniformly granular gemmules may be gathered from Pl. XX, Figs. 71 and 70, the latter showing only a small part of a gemmule with the neighboring tissues. Strange to say, in the *Tedania*s

which I preserved, in spite of the great abundance of full-sized gemmules, it was a very difficult matter to find medium sized and quite small ones. The only explanation I can think of is, that after a certain period no more gemmules are produced in the sponge, but those already formed are allowed to mature and develop into the swimming larva. In this way it might come about that in the latter part of the season a sponge should contain only mature gemmules, and I suppose it was towards the end of the season when my material was preserved. A few stages in the formation of the gemmule were, however, observed. A small gemmule is shown in Fig. 70 *g*. Its shape and the fact that it lies free in one of the canals indicate that it is amoeboid and was creeping about when killed. In structure it is precisely like the ripe gemmule, consisting of a finely and uniformly granular mass, the granules taking a deep stain. No cell boundaries nor nuclei were visible. Some very small masses, consisting of the same finely granular material and in which again no nuclei nor cell boundaries could be made out, were occasionally found imbedded in the mesoderm, Pl. XX, Fig. 69 *g*. These, from their histological similarity to the older stages, were construed as very young stages in the formation of gemmules. Now it will be remembered that in the mature gemmule of *Esperella* the cells are so closely packed and are so full of fine yolk granules, that the cell boundaries are very indistinct and the appearance is given to the gemmule of a uniformly granular mass with nuclei scattered through it, the nuclei being so small as to look like mere chromatin masses. This is what I suppose to be the true structure of the *Tedania* gemmule. I take it to be a mass of mesoderm cells in which the cell boundaries, owing to the compression of the cells and abundance of yolk, and the nuclei, owing to their small size, are obscured.

In the mature gemmule a few spicules or pieces of spicule are usually found, Fig. 70, *sp.*, which undoubtedly have not been formed in the gemmule itself, but have got in from the maternal tissue. The sheath or capsule which surrounds the gemmule is made up of closely packed fibre-like cells, *g. sh.*, Figs. 70 and 73. It is probably formed much as the corre-

sponding structure in *Esperella*, by the compression of the surrounding tissue owing to the growth of the gemmule.

Development of Gemmule into Swimming Larva.—The mature gemmule breaks up into masses, and these into smaller masses, and so on until the entire gemmule has been resolved into distinct cells, as in the development of *Esperella*. The process is odder and more striking looking in *Tedania*, owing to the absence of any indication of the individual cells and owing to the extreme irregularity of the first fissures, Fig. 72 (section through a gemmule just beginning to break up). In Fig. 73 is shown a portion of a section through another gemmule which has already broken up into small masses of varying size, even in the smallest of which nuclei are as yet invisible. In a stage a little later, Fig. 74 (the entire embryo was spheroidal), nuclei make their first appearance. In this embryo the division of the gemmule masses has been carried so far that the individual cells are easily recognisable. The superficial cells are packed tightly enough to make a continuous layer, which will become the ectoderm, inside which are scattered cells and rounded masses, separated by a clear fluid and more or less united by delicate protoplasmic processes. The bodies of the cells and rounded masses are just as full of the finely granular yolk as was the mature gemmule, and nuclei are only visible in some of the cells and a few of the masses. The masses are usually divided into rounded lobes and are obviously about to split up into individual cells.

The hitherto spherical embryo begins to assume an oval shape. In Pl. XXI, Fig. 75, is shown part of a section through a roughly oval embryo, in which the differentiation of the layers is noticeably more advanced than in Pl. XX, Fig. 74. Nuclei are apparent in all the cells, and the ectoderm is more distinctly marked off from the inner mass of cells (mes-entoderm). In some of the ectoderm cells two nuclei can be seen, indicating that cell division is taking place. The mes-entoderm in this stage consists of a very loose network of cells connected together by long slender protoplasmic processes.

Many of the mes-entoderm cells are closely packed in dense groups, and there are a number of multilobed and often multi-

nucleate masses, as in the previous stage. Most of the mes-entoderm cells have, like the ectoderm cells, plump bodies full of fine yolk granules, but there are some with smaller slender bodies, in which there is but little yolk and which begin to assume the appearance of the spindle-shaped cells, so abundant in the older embryo (compare Pl. XXI, Fig. 81). The pieces of spicules found here and there in embryos of this stage were already present in the gemmule before it began to break up. It will be noticed in this stage, Fig. 75, that the ectoderm cells are, in many instances at any rate, connected with the mes-entoderm cells by fine terminal processes. This connection probably continues to exist in the later stages, but I did not satisfactorily demonstrate it.

The ectoderm cells which already form a distinct layer in Fig. 75 divide in planes vertical to the surface, and become long slender columnar cells. These slender columnar cells form for a time a uniform investment for the whole embryo, Pl. XX, Fig. 76, though later they flatten out over one pole. In Pl. XXI, Fig. 76, is shown a small part of Fig. 76, more highly magnified. The rounded multilobed masses of the earlier stage are no longer found in the mes-entoderm, which now consists only of separate cells.

While the embryo is still in the body of the mother and surrounded by its capsule, the ectoderm cells over one of the poles flatten out, while elsewhere they develop cilia and become deeply pigmented. In Pl. XX, Fig. 77, is shown a section through this pole of the embryo at a stage just before the flattening has begun. The ectoderm cells over the general surface have flagella, and nuclei near their lower ends, the nuclei forming a zone several layers thick. The ectoderm cells at the pole, however (*ec. un-p. p.*), are not quite as slender and have no flagella. Their connection with the cells of the mes-entoderm is still obvious. These cells gradually flatten until, by the time the embryo leaves the body of the mother, they have assumed the character shown in Pl. XXI, Fig. 78 (*ec. un-p. p.*). The mes-entoderm in the stage shown in Fig. 77 is much as in the earlier stage, with the exception that a number of spicules are now scattered through it, all of them

very short and slender, and pointed at both ends (oxeate microcleres).

Structure of the Swimming Larva.—The larva, when it escapes from the body of the mother, is solid, of an oval shape, with one unpigmented unciliated pole, the rest of the body being covered with cilia and of a bright red color. It moves rapidly about in the water, occasionally creeping, but usually swimming, and it seems especially fond of making series of long shallow dives, coming up to or near the surface between the dives. The swimming larva can also change its shape to a slight extent.

The general ectoderm of the larva is composed of very long and slender cells, *ec.*, Pl. XXI, Fig. 78 (section through the unpigmented pole of a larva just born), which contain the bright red pigment. Each of these cells has a single flagellum, and the nuclei contained in their inner ends make a broad, deeply staining zone. The extreme peripheral ends of the cells are modified to form a cuticle, *cu.* in Fig. 78. The columnar ectoderm cells become shorter towards the unpigmented pole, as is shown in the figure, and yet pass with considerable abruptness into the flat cells covering this pole. The latter cells do not contain pigment, but are granular and stain deeply. The ectoderm remains unchanged during the free larval life (comp. Pl. XXI, Fig. 81, longitudinal section through a swimming larva a day old).

Like the ectoderm, the parenchyma of the swimming larva remains essentially the same throughout larval life—compare the two sections, Fig. 78 (through larva just born) and Fig. 81 (larva a day old). The parenchyma of the larva is much more differentiated than it was in the stage shown in Fig. 77. The parenchyma cells of the latter stage were essentially alike and were pretty evenly distributed, but during the last period of embryonic life, they become variously modified. Some of them crowd into the unpigmented end of the larva, becoming more and more tightly packed, and forming ultimately a dense mass of closely appressed polygonal cells, which stain faintly and the cell outlines of which are distinguished with difficulty, *p. c.*, Fig. 78. (In regard to this mass of cells, as in so many other

points, the larvae of *Tedania* and *Esperella* are essentially identical.) Next after the mass of pale cells comes an aggregation of large granular well-staining cells, *gr. c.*, Figs. 78, 81. Following upon the granular cells, the axial part of the larva, *ax. p.*, Fig. 81, is occupied by a mass of slenderer and less granular cells, provided with delicate processes, many of the cells being bipolar. This part of the larva is considerably denser than the peripheral part, *per. p.*, in which the cells are relatively less numerous, many of them lying in a more or less radial direction, parallel with the short spicules, which in the swimming larva are confined to this region. Besides the cells mentioned, there are found scattered here and there through the body of the larva a small number of *very* coarsely granular, deeply staining cells. A few of them are shown in Fig. 78, lying amidst the pale cells at the end of the larva; others are shown in Fig. 81, some of them in the ectoderm, others in the axial part of the larva. As in the case of *Esperella*, so in this larva, there is a loose bundle of long spicules in the unpigmented end of the body, Figs. 78 and 81. The spicules are the tylotes with nicked heads. The strongyloxeas, the spicules which in the adult form the skeletal meshwork, do not appear in the swimming larva.

There is only one noticeable change which occurs in the larva during its short swimming life, and which concerns the unpigmented pole. When the larva is just born, this pole does not protrude to any great extent, Fig. 78. Indeed, quite often this end of the body is pulled in, Fig. 79 (surface view of larva just escaped from parent sponge). But after fifteen or twenty hours of larval life, it is found that the unpigmented end protrudes to such an extent, that it is a very conspicuous feature of the living larva, Fig. 80. (Surface view of a larva a day old. In this figure the peripheral zone of short spicules is shown.) In sections, too, this difference between larvae just born and older ones, is noticeable—compare Figs. 78 and 81.

3. METAMORPHOSIS.

Attachment.—With most individuals the swimming life lasts about a day, with some two and three days. Towards the end

of the period, whatever it may be, the larvae become sluggish and lie about on their sides on the bottom of the dish. They then attach, the columnar ectoderm cells are transformed into flat cells, and the body of the larva flattens out into a round cake-like mass. The distinction between the pigmented and unpigmented portions of the body is entirely lost, the whole surface becoming red. In Pl. XXI, Fig. 84, is shown a surface view of recently attached sponge, and in Pl. XXII, Fig. 92, one-half of a vertical section through the same. The long spicules, which form a bundle in the unpigmented end of the larva, become scattered irregularly through the body. In attaching, almost all the larvae I have watched have stuck fast by their sides and not by one end. Such a larva just attached is shown in Pl. XXI, Fig. 85. Its outline still recalls the outline of the swimming larva (Fig. 80), it not yet having assumed the circular shape of Fig. 84. The body is solid; the ectoderm is entirely composed of flat and very thin cells; and the unpigmented or spicular pole of the larva (*sp. p.*) can still be identified both by the absence of pigment and the presence of the long spicules. The line of demarcation between the pigmented and unpigmented regions is not a sharp one, as it was in the swimming larva, and the spicules are no longer arranged in a bundle, but have begun to scatter about, though as yet they are still confined to one end of the sponge.

The attachment may take place obliquely, so as to bring the spicular pole on the upper surface of the metamorphosed larva, though near the periphery. In a few cases I have seen the attachment take place by the non-spicular pole, a little obliquely, to be sure, as is shown in the surface view, Fig. 82. In this larva the spicular pole was pulled in to such an extent, that at first sight it looked like an opening leading into the interior, though as a matter of fact it was nothing of the kind. When the attachment takes place by the end, as in Fig. 82, the spicular pole comes to occupy a more or less central position on the upper surface of the metamorphosed larva. In Pl. XXI, Fig. 83, is given a vertical section through a little sponge, which must have attached by the non-spicular pole, for on the upper surface and more or less in the centre is found the

ectodermal area (*ec. un-p. p.*) which covers the spicular pole in the swimming larva. These cells of the swimming larva, as has been said, stain very intensely, and have too characteristic an appearance for their identity to be doubted. The rest of the ectoderm in Fig. 83 is composed of flat, thin cells, but the area in question has precisely the same appearance as in the swimming larva. The long spicules too have remained about in the same position which they occupy in the swimming larva, pieces of them being shown in the figure directly beneath the deeply stained patch of ectoderm. The variation which the *Tedania* larvae exhibit in their manner of attachment, is shown in other silicious sponges (see section on Morphology of Sponges, p. 364).

That the columnar ectoderm of the larva flattens and is not cast off, is evidenced by the fact that during the metamorphosis the sponge retains its smooth surface, and that no membrane or bit of membrane is seen to be sloughed off. The flattening of the ectoderm takes place quickly, being completed a very short time after the fixation of the larva. In Fig. 83 the flattening of the columnar cells has taken place. After the polar ectoderm (*ec. un-p. p.*) has in like manner flattened, the entire investing layer of cells is so thin that it is best described as a nucleated membrane, *ec.*, Pl. XXI, Fig. 89.

During the flattening of the ectoderm the parenchyma of the larva also undergoes changes, as may be seen on comparing Figs. 81 and 83. In the attached sponge there are two kinds of cells which have no definite arrangement. There are first, great numbers of very small cells, so small that only the nuclei are seen with distinctness, the cell outlines being practicably indistinguishable; and there are also numbers of deeply staining, plump-bodied, granular cells, such as were found in the spicular end of the swimming larva. The sponge shown in Fig. 83 (vertical section) flattens out considerably, especially at its periphery, and assumes the shape indicated by the vertical section, Fig. 92, and the surface view, Fig. 84, the parenchyma remaining practically unchanged. With regard to the rearrangement of the spicules of the swimming larva, something has already been said of the long (tylote) spicules.

They become irregularly distributed through the body of the sponge. The short spicules (microscleres) likewise become distributed through the sponge body (Fig. 83), though the majority of them retain their peripheral location, many projecting from the surface of the sponge, as shown in Figs. 84 and 85. Sometimes a sponge is found with the peripheral microscleres arranged in as noticeably radial a fashion as in the swimming larva. Such an instance is shown in Pl. XXI, Fig. 86. As a rule the radial arrangement of the microscleres is not nearly so conspicuous as in this figure. At this period of its existence (Fig. 84), the sponge is a much simpler organism than during its swimming life, consisting as it does of a solid mass of parenchyma cells, in which there is no nice arrangement as in the free larva, and of an ectoderm which is nothing more than a nucleated membrane.

Ectodermal Membrane and Peripheral Mesodermic Zone.—After attachment the edge of the sponge is for a time more or less circular and smooth, and the mesoderm extends quite to the periphery, Pl. XXI, Fig. 84, and Pl. XXII, Fig. 92. The contour of the sponge then begins to change, and the periphery becomes more or less lobed and irregular, Pl. XXI, Fig. 86 (compare also the ectodermal outline, *ec.*, of the sponge given in Fig. 88). An accumulation of fluid then takes place in the extreme peripheral part of the sponge, by which means the ectodermal edge is pushed out some little distance from the edge of the mesoderm. In Fig. 88 this separation has taken place on opposite sides of the sponge, and between the edge of the mesoderm (*mcs.*) and that of the ectoderm (*ec.*) is seen a clear space occupied by fluid alone. In Fig. 89 is represented a small part of the periphery of a sponge in which this process is going on—the parenchyma cells are separated from the ectoderm much farther in the middle than at the sides of the figure. In Pl. XXI, Fig. 87, a portion of the periphery of another sponge, fluid separates ectoderm from mesoderm in the regions *a*, *b*, *c*. The ectoderm continues to grow peripherally, the distance between its edge and the edge of the mesoderm continually increasing. In this way the sponge body comes to be surrounded by a purely ectodermal membrane. In the

immediate neighborhood of the mesoderm, as is shown in the section, Pl. XXII, Fig. 94, the membrane, *ec. mem.*, consists of two layers, the upper and lower ectoderm respectively, but farther out it is one-layered. The ectodermal membrane extends for some distance beyond the body of the sponge, and is more or less covered with debris. It is essentially like the corresponding structure in *Esperella*. The membrane is shown in sections in Fig. 93, and in the surface view, Fig. 90, its outline, *ec. mem.*, is partly indicated. The sponge shown in Fig. 90 is only partially surrounded by the ectodermal membrane, retaining its earlier character in the region *a*, where the ectoderm has as yet taken no step towards forming a membrane. Nuclei could be made out here and there in the membrane and in the ectoderm proper, but the cell outlines I could not distinguish. The same deeply staining thickenings which were found in *Esperella*, are again found in the basal ectoderm and membrane of this sponge, *pr. th.*, Pl. XXII, Figs. 93 and 94. The only construction to be put upon them seems to be that they are nuclei surrounded by protoplasm.

As the ectoderm grows out to form the membrane, the peripheral mesoderm throws out lobes and processes, its outline becoming jagged and irregular, as in Fig. 88, *mes.* The cells of this part of the mesoderm gradually form a peripheral zone, distinguishable from the rest of the body by the fact that they are much less closely packed than the cells elsewhere (*p. z.* in Pl. XXII, Figs. 90, 91, 93). The cells of this peripheral mesodermic zone develop slender processes, and form a net-work (sections, Figs. 93 and 94, *p. z.*), which, however, is not nearly so open and exquisite as in *Esperella*.

During the formation of the ectodermal membrane, and afterwards during all the time I kept the young sponges, they underwent an incessant change of shape, which was more conspicuous during the first three or four days than it was later. This change of shape, though gradual, was greater and more rapid than in *Esperella*, and the little sponges were much disposed to assume peculiarly irregular shapes, such as that of the sponge shown in Fig. 91 (*mes.* indicates outline of the parenchyma — the whole sponge is supposed to be surrounded

by an ectodermal membrane). The change of contour concerns especially the parenchyma, which pushes out lobes and processes inside the ectodermal membrane, thus acquiring from time to time entirely different outlines, while the surrounding ectodermal membrane remains practically unchanged. If the parenchyma, however, continues to change its contour in such a way that the shape of the whole sponge is altered, as, for instance, in passing from a circular outline to a shape such as that in Fig. 91, then the ectodermal membrane is involved and its edge gradually altered so as to remain more or less parallel with the general contour of the parenchyma.

In sponges which have assumed elongated irregular shapes, like that of Fig. 91, the change of contour sometimes leads to the complete division of the body into two independent sponges. This phenomenon I have twice observed. I thought at one time that I had witnessed the converse phenomenon, *i.e.* the fusion of two attached sponges into one. I observed two sponges, a couple of days after attachment, which lay near each other, grow nearer and nearer until after fifteen to twenty hours they met and seemed to fuse into one body of an irregularly oval shape. Across this body, however, could be seen the seam or line of fusion, and the union must have been one of close juxtaposition only, for after a few hours the sponges again separated along this line and afterwards remained independent. Fusion of the swimming larvae, into a single large one, as occasionally happens in the Coelenterates (*Manicina*) I have never observed.

Canal System. — The canals and subdermal cavities appear as separate lacunae in the parenchyma, the surrounding cells becoming modified into epithelioid membranes, Figs. 93 and 94, *s. d. c., can.* The separate lacunae subsequently become united into a canal system, as in *Esperella*. The flagellated chambers likewise originate as independent structures, which later acquire connection with the canals, Fig. 93. The subdermal cavities, which in some cases are very extensive, as in Fig. 91, *s. d. c.*, are roofed over by a dermal membrane (*d. mem.* in Fig. 93), quite like the same structure in the young *Esperella*. As in *Esperella*, no system is followed in regard to

the order of formation of the canals. In some individuals the first cavities formed are narrow rounded canals, *can.*, Pl. XXII, Fig. 88, covered in only by the dermal membrane, and which from the surface look like oscula. Occasionally a stage is found where but one of these canals exists, and that in the centre of the body, Fig. 90. Such a stage is interesting, because of its essential resemblance to the young *Reniera* (Marshall), or *Chalinula* (Keller), etc., in which sponges the first canal to form is regularly a main central cavity which is ordinarily homologised with the central cavity of calcareous sponges and regarded as the gastrula cavity.

Pores and oscula were developed in only a few of the sponges I reared, and were themselves few in number. They made their appearance without order, scattered about as in *Esperella*, and in other respects too their formation agreed with that of the same structures in the latter sponge.

III. ADULT STRUCTURE AND EGG DEVELOPMENT OF *TEDANIONE FOETIDA*, N. G.

I. ADULT.

It is necessary to create a new genus for this form, which, it would seem, however, is closely related to *Tedania*. The spiculation of the two genera separates them, though the occasional presence of tylotes in *Tedanione* coupled with the great similarity in the canal system and histological structure makes a close kinship between the two very probable.

Diagnosis of Genus.—Spicules mostly oxeas, with microscleres of same pattern, and a very few tylotes. Flagellated chambers open directly into afferent and efferent canals.

Tedanione foetida, n. sp.—Sponge amorphous with two or three cylindrical oscular papillae one inch high. Size, rarely over three inches from osculum to base. Sponge is slatebrown, has a fetid odor in life, and the surface is irregularly and inconspicuously furrowed. Main efferent canals surrounded by large amount of gelatinous tissue which only occasionally comes to the surface. Pores rare and scattered. Subdermal cavities

everywhere beneath a dermal membrane. Spicules, stout skeletogenous oxeas, $\frac{3.5}{100}$ mm. long and often slightly bent; microscleres (oxeas) of varying length; also a few tylotes. Dermal membrane strewn with oxeas of full size, amongst which are scattered microscleres, with here and there a tylote. Membrane supported by brushes of oxeas containing a very few tylotes. Spongy tissue contains radial skeletal bundles, composed of oxeas, running in from the brushes; bundles composed of same spicules crossing the former at right angles some little distance below the surface; and numbers of oxeas scattered freely through the tissue in such a way that they cross one another in every direction, but are not cemented together to form a network. Gelatinous tissue contains both ordinary oxeas and microscleres scattered freely about, microscleres most abundant immediately round main efferent canals. *Green Turtle Cay, Bahamas.*

Tedanione foetida is found in the "sounds" on the roots of the mangrove. The surface of the sponge is furrowed in a manner recalling the surface of *Tedania*, but the furrows are not nearly so abundant nor conspicuous as in the latter genus. On cutting the sponge open it is seen that the body is divided as in *Tedania* into spongy and gelatinous tracts, the gelatinous tissue lying around the main efferent canals. But it is only occasionally that the gelatinous tissue comes to the surface. In most places it lies in the interior completely covered by spongy tissue. In Pl. XXII, Fig. 96, a vertical section through the base of the sponge is shown, and it is seen that the gelatinous tissue is wholly in the interior of the body. In Pl. XXII, Fig. 95, a transverse section through an oscular papilla is shown, and here the resemblance to *Tedania* is greater, for the gelatinous tissue comes to the surface in several places.

The dermal membrane is strengthened by numerous oxeas of full size, Pl. XXIII, Fig. 100, and the pores are few and scattered. Pl. XXII, Fig. 97, represents a section vertical to the surface, and shows the gross features of the canal system. The subdermal cavities are numerous and open into larger or smaller afferent canals, by which the water is introduced into the spongy regions. The flagellated chambers communicate

directly with the afferent canals on the one side and the efferent canals on the other, as may be gathered from Pl. XXII, Fig. 98, representing a small portion of the mesoderm of the sponge. Efferent canals are abundant round the edge of the spongy regions (Figs. 95 and 97), and communicate with the larger efferent canals lying in the heart of the gelatinous tissue. In the body of the sponge the main efferent canals pursue an irregular course, but in the oscular papillae they run longitudinally, there being at the base of the papilla several which gradually run into one another as they near the summit of the papilla. There is usually one canal in the axis of the papilla, which is larger than the rest and may be considered the main canal of the papilla.

The gelatinous tissue is much like that of *Tedania*, consisting of a network of cells with a watery jelly in the meshes. As in *Tedania*, there is an abundance of delicate bipolar cells, the processes of which are long, slender, and branching. There are also numerous large granular cells, not present in *Tedania*. Pl. XXIII, Fig. 99, is a small portion of a section showing the gelatinous tissue lying between two canals (*c. w.* = canal wall).

The general arrangement of the skeleton is shown in Pl. XXII, Fig. 97. The brushes of spicules supporting the dermal membrane, the radial and tangential bundles, and the distribution of the free spicules, need no further description. Where the gelatinous tissue comes to the surface, the brushes of spicules supporting the dermal membrane are either absent, or feebly developed. The skeletogenous oxea is very often found with its two ends modified after the fashion shown in Pl. XXII, Fig. 101. The length of the process *x* varies considerably. What few tylotes occur are found either in the dermal membrane or in the brushes supporting it. I have seen three or four tylotes with nicked heads like those of *Tedania*. As in *Tedania*, the microscleres are most abundant round the walls of the efferent canals, but while they are larger than the microscleres of *Tedania*, they are much less numerous. It may be mentioned that after hunting persistently through many sections and caustic potash preparations of this sponge, I have found four anchors, varying in size but otherwise alike. Being unable to

find any more, my conclusion is that these anchors are foreign particles, and that bits of the sponge to which they originally belonged entered Tedanione and were used as food.

2. DEVELOPMENT.

My observations on the development of Tedanione and *Hircinia* deal only with the egg development, going in the former sponge as far as the formation of the swimming larva, but in the latter no farther than the segmentation.

Tedanione was with eggs in September and October and possibly for a much longer time at Green Turtle Cay, Bahamas. Adults were kept in aquaria, and after an hour or two, as a rule, a few ciliated larvae were thrown out of the oscula.

The very young ovarian egg is of an irregular shape and lies in the mesoderm surrounded by a follicle of flattened cells, Pl. XXIII, Fig. 102, *ov. o.* It has a large nucleus and single nucleolus. As the egg increases in size it becomes rounded, its protoplasm becomes filled with yolk, and the nucleus undergoes certain changes, which are not completed until the egg has attained its full size and is ready for segmentation. A general idea of the change in size and character of the egg during its growth may be gathered from a comparison of Pl. XXIII, Figs. 102, 103, 104, 105, 106, drawn to the same scale and representing successive stages in the life of the egg.

During the increase in the size of the egg, its follicle is constantly surrounded by a dense mass of mesoderm cells, as may be seen in the section, Fig. 103 (*mcs.* = the cells in question; the egg is one-half the full size). These cells have large, plump bodies which stain well, being full of a finely granular yolk. The shape and direction of the cells on the outskirts of the mass indicate a migration of mesoderm cells from all quarters to the egg. By the time the egg has reached its full size the surrounding mass of cells has dwindled away to a small number, Fig. 104, and during the remaining period of its life in the parent sponge the embryo is surrounded by ordinary mesoderm, in which the cells are not more thickly crowded than elsewhere in the body. It is only while the egg

is growing larger and becoming stored with yolk that it is surrounded by the cells in question, the purpose of which it would seem is to bring food to the young egg. Since none of the surrounding mesoderm cells are ever seen to break through the follicle, it must be that the food is passed through the follicular membrane in a liquid shape and is then absorbed by the egg. Fiedler's description (5) of the manner in which nutrition is brought to the growing egg of *Spongilla*, differs from the above account in some respects. In *Spongilla* special "Nährzellen" congregate round the egg during its growth, and penetrate between the follicular cells, supplying the egg with food. The "Nährzellen" do not fuse with the egg, and it would seem that the food must be transferred by osmosis. As in *Tedanione*, the nourishing cells disappear when segmentation begins.

The very small ovarian egg is filled with the extremely fine granular yolk which is found in the body of any mesoderm cell at all noticeable for its size. But as the egg increases in size, yolk of a different character makes its appearance, consisting of small spheres thickly packed. In eggs of about one half the adult size, Fig. 103, these small spheres may be found filling the entire peripheral region, but leaving round the nucleus an area containing only fine granules. With continued increase in size the whole egg becomes filled with yolk spheres, which themselves increase considerably in size, as may be seen on comparing Fig. 103 with Fig. 104, the egg in the latter figure being of full size.

The nucleus of the young egg cell contains a single nucleolus which occupies a more or less central position, Fig. 102. By the time the egg has reached a size equal to one half that of the ripe egg, the single nucleolus has given place to two, which are invariably placed on opposite sides of the nucleus and adhere to the inner surface of the nuclear membrane, Fig. 103. In eggs which have reached the adult size it is the rule to find either one nucleolus peripherally placed, as in Fig. 104, or the nucleus contains no nucleolus at all, as in Fig. 105. It sometimes happens that an egg of full size is found with two nucleoli, but this is rare. From this evidence it would seem that

the two nucleoli present in the developing egg are lost, one after the other, at the time when the egg reaches its full size. As to how the first of the two is lost, I have no evidence, but the second nucleolus may often be seen lying just outside the nucleus in the yolk, Fig. 105 *n''*, showing that it has been extruded from the nucleus. The nucleus differs in size so little from the yolk balls, and the latter stain so deeply, that I was at first in doubt whether to claim the object seen just outside the nucleus as an extruded nucleolus, or to regard it as merely a yolk ball. But so many eggs showed this one very deeply staining sphere in about the same position, that I was finally convinced it could be nothing less than the extruded nucleolus. Very rarely an egg much less than the full size is found with but a single peripherally placed nucleolus, indicating that the first nucleolus has already been lost. But this is a rare exception, the rule being that the nucleoli disappear only after the egg attains its full size. The nucleus which remains after the extension of the nucleoli, Fig. 105, has a membrane and finely granular contents which stain feebly.

My observations on the formation and loss of the nucleoli were made in the Bahamas in the fall of 1888. On my return I found that Fiedler (5) had just described the same phenomena in *Spongilla*, and regarded the two small nucleoli as polar bodies. Fiedler finds that the two small nucleoli are constricted off as buds from a larger central one, the latter remaining after the extrusion of the former. Further, at the time when the nucleoli are extruded, the nuclear membrane disappears. There are thus some differences of detail between our accounts. In the interpretation of these bodies as polar globules I cannot agree with Fiedler, because they are formed (though not discharged) long before the egg reaches its full size. Moreover, polar bodies of the ordinary metazoon type exist within the group of sponges, as is shown by Magdeburg's discovery of them in *Plakina trilopha* (see the notice of Magdeburg's unpublished observations in Korschelt & Heider, p. 1).

The segmentation of the egg of *Tedanione* is total, and regular, at any rate as regards the first two planes. In Pl. XXIII, Fig. 106, is shown the stage of two segments, and in Fig. 107

the stage of four. In these early stages the nuclear membrane could not be made out, the clear space round the nucleolus being only vaguely outlined. In a later stage, Fig. 108, probably sixteen segments, the membrane first makes its appearance, though it has no doubt been there the whole time. An advanced stage of segmentation is shown in Fig. 109, a still later stage in Fig. 110, an embryo as yet unciliated in Fig. 111, and the ciliated free swimming larva in Fig. 112.

In Fig. 110 is shown a curious phenomenon. What appear to be cell membranes are distinctly seen round many of the segments, the body of the cell in some cases having fallen out of its surrounding membrane. These membranes are protoplasmic and are undoubtedly artefacts caused by the fixing fluid. They are of interest only as indicating how sharp the demarcation is between the cortical layer of pure protoplasm which invests each segment, and the yolk-containing protoplasm which makes up the mass of the segment. In the sudden contraction due to the stimulus of the fixing fluid, it would appear that the central and cortical protoplasms of one segment part company more easily than the cortical layers of adjacent segments, which in life must be closely appressed. Though I regard these "membranes" as artefacts, I am aware that Schulze has described somewhat similar structures in the larva of *Euspongia* (37), which he considers to be of a normal nature. Between the parenchyma cells of the solid larva of *Euspongia*, Schulze describes partitions, which he is in doubt whether to regard as secretions or as the modified cortical layers of the cells. He thinks it probable that they are later transformed into the uniform watery jelly in which the cells lie, and points out the analogy to cartilage, comparing the intercellular partitions with the capsules which go to form the cartilage matrix.

The cells of the embryo, Fig. 111, are full of fine yolk, and, being very closely appressed, the outlines are indistinct as compared with earlier stages. The metamorphosis of the large yolk spheres of the ripe egg into fine yolk goes on gradually during the segmentation (compare Figs. 104, 106, 108, 109, 110, 111), retracing the path which was followed in the development of the small egg cell into the ripe ovum.

In the transformation of the simple embryo, Fig. 111, into the ciliated larva, the outer cells become the ectoderm, the inner cells forming an undifferentiated mass, the parenchyma or mes-entoderm. When the embryo escapes from the parent and begins its free swimming life, it is in the condition shown in Fig. 112 (longitudinal section). The ciliated larva is of an oval shape, one end being considerably broader than the other, and of a uniform brown color. It is ciliated all over, there being no differentiation of an unciliated, unpigmented pole as in the gemmule larva of *Tedania* and *Esperella*. I did not follow the further development of the swimming larva, but it is quite possible that an unciliated pole may later make its appearance, as in the *Desmacidon* described by Barrois.

The ectoderm of the larva is uniformly composed of very long slender cells, the peripheral ends of which contain the nuclei and being free from yolk form a zone clearly marked off from the rest of the larva, Fig. 112. In this zone the outlines of the ectoderm cells are plain enough. The ectoderm cells, however, extend a long distance internally from this zone, and their inner portions containing fine yolk, similar to that with which the mes-entoderm cells are filled, the cell outlines are here not very distinct. The parenchyma at first sight appears to be a uniformly granular matrix containing nuclei. But in very thin sections its cellular nature can be made out. It is composed of irregularly polygmal cells, which are so closely appressed and so full of fine yolk granules, that the cell boundaries are obscured.

In sectioning the parent sponge for embryos, I came across the curious case of attachment illustrated in Fig. 113. A ciliated larva of an irregular shape, and containing two or three flagellated chambers, is present in one of the larger canals, and appears to have attached to the wall of the canal instead of passing out of the body of the mother. There is, as can be seen in the figure, a perfect continuity between the mesoderm of the parent and the parenchyma of the larva. The columnar ectoderm on the other hand does not seem to be continuous with the epithelioid lining of the canal, but rather to pass into the mesoderm of the adult through a break in the canal wall.

There are two or three flagellated chambers present in this sponge, one of which is shown in the figure, *f. c.*, but I can communicate nothing as to the details of their formation, except that they are independent of one another and surrounded by a solid mass of cells.

IV. EARLY STAGES IN EGG DEVELOPMENT OF *HIRCINIA* ACUTA.

The "Loggerhead" sponge, *Hircinia acuta*, is very abundant in the shallow water round Green Turtle Cay, forming circular masses often of very large size, which contain great numbers of annelids, *Alpheus*, and other semi-parasitic forms. It is with eggs in this locality during September, and probably for a much longer period. My observations on this form are very few, dealing only with the development of the ovarian egg and the segmentation.

The mesoderm of *Hircinia* has in many regions a cartilaginous appearance, consisting of a clear non-stainable matrix containing cavities in which lie the cells. When the cell shrinks away from the wall of the cavity, the latter comes plainly into view, Pl. XXIII, Fig. 114 (bit of the mesoderm). Tracts of this sort are often found in which flagellated chambers are absent, and in such places egg cells frequently occur. In Fig. 114 is shown an egg-cell (*o. ov.*) about twice the size of the surrounding mesoderm cells, and containing a large nucleus with a single nucleolus. The egg is enclosed by an incomplete follicle, composed of neighboring mesoderm cells which apply themselves closely to the wall of the egg cavity. During its increase in size the egg becomes stored with yolk, and its nucleus undergoes changes similar to those described for *Tedanione*.

To the few mesoderm cells enclosing the young egg, others are gradually added, and in this way a complete follicle is formed, Pl. XXIII, Figs. 115, 115¹, consisting of a single layer of cells. In *Tedanione* the follicular cells very early flatten out into a thin membrane, but in *Hircinia* this change does not take place until after the beginning of segmentation. In

Hircinia there is the same indication as in Tedanione, that the mesoderm cells bring food to the growing egg. Until segmentation begins the egg is thickly surrounded by mesoderm cells, Pl. XXIV, Figs. 116 and 119, which have large bodies full of fine yolk granules. Amongst these cells are scattered a few with very coarsely granular bodies, Fig. 119. The mesoderm cells are applied so closely to the follicle that the latter cannot be distinguished as a definite layer. All that can be said is that at this time the egg is surrounded by closely packed cells, arranged irregularly in two or three strata, the inner stratum doubtless representing the follicle of the very young egg, while the outer strata consist of mesoderm cells which have applied themselves to the follicle. The inner stratum of cells is, as may be seen in Fig. 116, often very irregular, and there are certain indications that the follicular cells are sometimes pinched off and engulfed by the egg — notice the very protuberant rounded cells projecting into the substance of the egg. Whatever be the precise manner in which food is conveyed from the surrounding cells to the egg, it seems pretty certain that food is so conveyed, and that this is the object of the migration of so many mesoderm cells to the immediate neighborhood of the growing egg. The egg at the time when it reaches full size is still surrounded by several strata of cells, Fig. 119. The inner stratum, however, soon becomes transformed into a follicular membrane, consisting of flattened but still rounded and protuberant elements, Pl. XXIV, Fig. 120. The cells of the outer strata gradually wander away, leaving the mesoderm round the segmenting egg not more abundantly supplied with cellular elements than is the mesoderm in most parts of the sponge.

The ripe egg of Hircinia is closely packed with yolk spheres of a large size, Fig. 119, which make their appearance in the developing egg-cell after a fashion essentially similar to that already described for Tedanione. In the finely granular body of the very young egg-cell, yolk spheres appear which are at first small, Figs. 115, 115', but which gradually increase in size, becoming at the same time more closely packed. In the egg shown in Fig. 116, the bulk of the yolk is still composed

of quite small spheres, but scattered about in the fine yolk are certain large rounded bodies which I take to be the first large spheres formed.

While the egg is still small, long before it reaches the full size, the single nucleolus gives place to two nucleoli peripherally placed, as in Pl. XXIII, Figs. 115 and 115¹. The egg of Pl. XXIV, Fig. 116, has likewise two nucleoli, situated in the same way, but the section passed through only one of them. Between the two nucleoli there is a sphere of granular material, which stains much less deeply than the nucleoli themselves, and which is separated from the nuclear membrane by a clear space. When the egg attains its full size, one of the nucleoli is lost, leaving no perceptible trace behind. In the nucleus thus left with a single nucleolus, Fig. 117, the nuclear membrane could not be made out in my preparations, though the sphere of faintly staining granular material was obvious, Fig. 118 (portion of the periphery of such an egg as that of Fig. 117). The second nucleolus is then extruded, and may be seen lying in the yolk in the immediate neighborhood of the nucleus, which now consists exclusively of the sphere of granular material, separated from the yolk by a narrow clear space, Fig. 118.

Segmentation results in the formation of a solid morula. An early stage in the segmentation is shown in the section, Fig. 120, and two morulas are shown in Figs. 121, 122. In the latter morula one of the segmentation spheres has been retarded in its division, and is consequently of a much larger size than the rest. Scattered between the segmentation spheres and forming a layer round them, there will be noticed a peculiar granular stuff which stains feebly but which is very conspicuous in the sections. It is probably a precipitate from the fluid bathing the segmentation spheres, caused by the fixing fluid (Perenyi).

V. REMARKS ON THE MORPHOLOGY OF SPONGES.¹

I have shown that in *Esperella* and *Tedania* the subdermal cavities, canals, and chambers develop as separate lacunae in

¹ The figures which illustrate this article, excepting Fig. 5, are borrowed. For their sources see description of the plates.

the parenchyma or mes-entoderm of the attached sponge, subsequently becoming connected into a continuous system. As regards the development of the canal system, such varying accounts are given by different authors that, were it not for the help lent by comparative anatomy, it would be quite impossible to form any idea of the fundamental morphology of sponges. Fortunately for the student entering this puzzling domain, comparative anatomy has in the hands of Haeckel, Schulze, and Polejaeff provided a standpoint from which the varying phenomena of development and structure may be viewed with at least a partially understanding eye. It may be that an increasing accumulation of facts will show that Haeckel's conception of the relation of the simple calcareous sponges to the complex horny and silicious forms is not well founded, and that Schulze's view of the parts played by the embryonic layers in producing the adult anatomy is not the true one. But at present it is only with the aid of these theories that one can form any clear conception of sponges in general, and so for the present at least we are bound to accept them.

Comparative anatomy points in no undecided manner to the phylogenetic path along which sponges have developed, and so permits us to construct a standard of ontogeny, with which we may compare the actual development of each species as we witness it to-day, and so be enabled to note the amount and kind of divergence (coenogeny) exhibited. That coenogeny is exhibited to a great degree in the embryology of sponges is evident from the various types of development described, and in the future much may be hoped from the study of a group like this for the understanding of the laws of development. For the present all we can do is to accept what seems the most probable phylogeny, recording the instances of supposed coenogeny as they are observed. Adopting this method, I have to regard the development of *Esperella* and *Tedania* (*i.e.*, the later development or metamorphosis) as far removed from the phylogenetic path. Before pointing out the pronounced coenogeny exhibited in the development of these sponges, it will be worth while to review briefly the evidence on which rests the current view of sponge morphology.

Evidence from Comparative Anatomy as to Sponge Phylogeny.—The strongest evidence offered by comparative anatomy lies in the series of forms, passing by gradations from very simple to complex types, found in the calcareous sponges (Haeckel 8, Polejaeff 20), and in the little group of silicious sponges, the Plakinidae, described by Schulze (26). A comparison of these forms goes to show that the simple Ascon sponge (*Olynthus*) must be regarded as the ancestral type of the group, and that by the continued folding of the wall of this simple form were produced the more complicated sponges. Further, the exceedingly complex silicious and horny sponges must be interpreted as colonies in which the limits of the individual can in many cases no longer be recognized.

The calcareous sponges offer a series of increasingly complex forms, which Haeckel divided into Ascons, Sycons, and Leucons. Haeckel's views on the relationship of these forms must be in great measure accepted to-day, though in certain respects, especially as regards the anatomy of the Leucons, later researches (Polejaeff, *l.c.*) have shown that he was not always in possession of the real facts of the case.

The simplest calcareous sponges, or Ascons, which serve as the basis for Haeckel's hypothetical sponge ancestor, the *Olynthus*, are too familiar to call for any description. The interesting form *Homoderma sycandra* (von Lendenfeld) may, however, be mentioned, in which the body is surrounded by radial tubes, after the fashion of a *Sycandra*, but with the difference that the central cavity as well as the radial tubes is lined with collared cells. A figure of this interesting sponge is accessible, in Sollas's article on Sponges in the *Encyc. Brit.* (or *Zoölogical Articles* by Lankester, etc., p. 40).

Homoderma bridges the way from the Ascon type to the simplest Sycons, in which the radial tubes are distinct from one another. A surface figure of such a Sycon (*Sycetta primitiva*) is given in Vosmaer(33), Taf. IX, taken from Haeckel's monograph. In the majority of Sycons, however, the radial tubes are not distinct, but are connected together more or less by strands of mesoderm covered with ectoderm (Pl. XXV, Fig. 1, transverse section of *Anamixilla torresi*). In this

sponge all the tubes are connected together, and the canals lying between them (Intercanals, *In. can.* in the figure) are complicated. Water enters the intercanals through the openings on the surface (surface pores, *s. p.*), and passes into the radial canals through the numerous chamber pores (*c. p.*).

The embryology of the Sycons as far as known confirms the belief that they are derived from the Ascons. Thus *Sycandra raphanus* passes through a distinctly Ascon phase (Schulze 25), the radial tubes appearing later as outgrowths. The actual development of complicated intercanals such as those of *Anamixilla* has never been witnessed, but a comparison of a large number of forms in which the connection between the radial canals varies within wide limits, makes it pretty certain that the intercanals of a form like *Anamixilla* are homologous with the simple ectodermic spaces between the radial tubes of *Sycetta* or *Sycandra ciliata*. It is exceedingly probable that the actual development of the complicated Sycons will show that the radial tubes are in young stages distinct from one another, and only later become connected together by bridges of tissue in such a way as to form complex intercanals. And so, we must at present regard the intercanals of a form like *Anamixilla* as lined with ectoderm.

Coming now to the Leucons, we find that Polejaeff's description of the anatomy of this family accords with their derivation from the Sycons quite as well as did Haeckel's more imaginative conception of the structure of these forms. Taking one of the simplest of Polejaeff's types, let us compare it with a Sycon. In Pl. XXV, Fig. 2, is shown part of a transverse section of *Leucilla connexiva*. Such a form is obviously derived from a Sycon by the evagination of the wall of the paragastric cavity at certain points (x, x). These evaginations give rise to numerous diverticula of the central cavity, which constitute efferent canals, *ef. c.* The radial chambers are at the same time thrown into groups, each group opening into one of the new diverticula. The intercanals (*In. can.*) penetrate as before between the several radial chambers, bringing water to the chamber pores (*c. p.*), the complexity of their

arrangement naturally having been increased by the folding of the wall of the paragastric cavity.

The increasing complexity in the Leucon family is brought about by the ramification of the primitively simple efferent canals, the radial tubes growing shorter and becoming in the most complicated types spheroidal chambers quite like the flagellated chambers of the non-calcareous sponges. In *Leucilla uter*, for instance, of which part of a transverse section is given in Pl. XXV, Fig. 3, the efferent canals exhibit branching of a simple character. But in such a form as *Leuconia multiformis* (transverse section, Pl. XXV, Fig. 4), the ramification of the efferent canals becomes exceedingly complex, and the radial tubes here appear as spheroidal flagellated chambers. The intercanals (or afferent canals, as they are called in all sponges but the Sycons) follow the efferent canals in all their windings, bringing water from the surface pores to the pores in the walls of the flagellated chambers.

The chief conclusions to be drawn from this anatomical comparison of the various forms of Sycons and Leucons are, that the afferent canals of Leucons are homologous with the intercanals of Sycons and are lined with ectoderm; that the flagellated chambers are homologous with the radial tubes; that increasing complexity is brought about by the ramification (or folding of the wall) of the efferent canals.

The canal system of a complicated Leucon, like *Leuconia*, is essentially like that of a common silicious or horny sponge (having flagellated chambers, afferent and efferent canals), except in the one respect that in the Leucon there is a single central cavity opening by a terminal osculum, while in most silicious and horny sponges there are several oscula leading into as many spacious efferent cavities. But here the disposition of the calcareous sponge to form indubitable colonies helps us out, for if we compare the silicious or horny sponge with a colony of Leucons instead of with a single one, we find that its derivation from such simple symmetrical forms is made easy. Robbed of its details, a silicious sponge of the character of *Esperella*, *Tedania*, or *Tedanione*, exhibits a structure illustrated by the diagram of an hypothetical silicious

sponge shown in Pl. XXV, Fig. 5. In the section drawn, three main efferent canals (*ef. c.*) are shown, each with its osculum (*os.*) and its very irregular set of branches (*ef. c'*, *ef. c''*, *etc.*), on the walls of which open the flagellated chambers (*f. c.*). The pores on the surface of this sponge (*s. p.*) lead into wide chambers (*s. d. c.*), the so-called subdermal cavities, from which run the afferent canals (*af. c.*), carrying water to the pores in the walls of the flagellated chambers. The distinction between subdermal cavities and afferent canals is more or less artificial, for the sharpness with which they are marked off from one another varies within wide limits. They are both parts of the same system, the subdermal cavity being merely a main afferent canal, which is especially enlarged in a tangential direction. The water may enter the chambers in some cases directly from the subdermal cavities, but for the most part it is carried to the chambers by the afferent canals, which branch and twist about, following the irregular course of the efferent canals. The mesoderm between the two sets of canals is reduced to comparatively narrow trabeculae, in which lie the flagellated chambers, arranged in a much folded but still single layer. The spicules which are not shown in the figure are in the mesoderm, either scattered about or united into a meshwork or a series of bundles. The genital products are also to be found in the mesoderm, scattered about, as a rule, in any part of the body.

The structure of a horny sponge, such as the sponge of commerce (*Euspongia*), is essentially similar to that of the hypothetical silicious sponge I have just described. The differences concern especially the skeleton and the precise manner in which the flagellated chambers are connected with the canals. In the horny sponges the silicious spicules give place to a meshwork of horny fibres, which lie in the mesoderm between the canals to which they lend support, and to the course of which their arrangement is adapted. The flagellated chambers in *Euspongia*, as in many other horny and silicious sponges, do not open directly into spacious efferent canals (as in Fig. 5), but indirectly by means of special canals, one of which runs from each chamber. And so it is with the afferent canals,

which in these sponges send a special little canal to each chamber (see Schulze's figure of *Euspongia*. *Zeit. f. Wiss. Zool.*, Bd. XXXII, Taf. XXXVI, Fig. 2). The difference between the two canal systems is easily explained, that of *Euspongia* being derived from the type shown in Fig. 5, by the pushing out of minute diverticula from both afferent and efferent canals.

Having now obtained a generalised idea of a complex non-calcareous sponge, it will be found a simple matter to compare such a form with a *Leucon* colony, of which I give a surface figure, taken from Vosmaer, in Pl. XXV, Fig. 6. The structure of the silicious sponge is readily understood if we suppose it to be a colony, in which the limits of the individuals have been lost or obscured by the increasing thickness of the walls of adjacent individuals. This increasing thickness would finally result in a more or less complete fusion of the members of a colony into an undivided mass with oscula scattered over the surface. Each of the main efferent canals of the silicious sponge is homologous with the paragastric cavity of a single *Leucon*. Both the canal and its set of branches, though, are extremely irregular, having completely lost the symmetry of the ancestral type. The flagellated chambers, however, still bear the same relation to the efferent canals as they did in the *Leucon*, *i.e.* they are simple diverticula of the canal wall. The system of afferent canals is obviously homologous with the same system in the *Leucons*, bearing identically the same relation as in the latter group, both to the flagellated chambers and the efferent canals. The subdermal chambers, communicating with the exterior by numerous pores, though a late acquisition, are found in certain *Leucons*, *e.g.* *Eilhardia Schulzei* (Polejaeff, Pl. IX).

In many of the non-calcareous the colonial nature of the sponge is indicated by the presence of elevations (oscular tubes or papillae) bearing oscula on their summits. But the number of oscula is not always to be taken as indicating the number of individuals of which the sphere is composed, for the colonies of calcareous sponges show plainly that the budding individuals do not always develop oscula. And on the other hand there

are certain indications in the silicious sponges (p. 8) that in the adult, oscula may be developed almost anywhere. Such facts make it impossible to fix upon the number of component individuals in any sponge. Perhaps the nearest approach made in other groups to the formation of colonies, in which the personality of the component individual is so nearly lost, is found in corals like *Maeandrina*, in which the united gastric cavities of the polyps form continuous canals, perforated at intervals by mouths.

We therefore reach the conclusion that the higher sponges (Non-Calcareae) have been derived from colony producing, symmetrical forms, in which the evaginations of the primitively simple paragastric cavity had already taken the form of efferent canals and flagellated chambers, that is from forms allied to the existing *Leucons*. And further we come to the conclusion that the subdermal cavities and afferent canals are homologous with the intercanals of *Sycons*, and hence, phylogenetically at least, are infoldings of the ectoderm. The whole efferent system, canals and flagellated chambers both, on the contrary is homologous with the same system in the calcareous sponges, and is endodermic.

This conclusion as to the parts played by the germ layers in producing the adult non-calcareous sponge, is the one enunciated by Schulze in his classical paper on the *Plakinidae* (p. 438). In this little family of silicious sponges Schulze finds a genus, *Plakina*, the three species of which form links in a chain of increasing complexity, showing quite as plainly as do the calcareous sponges that the afferent system is derived from ectodermal infoldings, and the efferent from endodermal outfoldings.

The *Plakinidae* are *Tetractinellids*. The three species of *Plakina* are small encrusting sponges found in the Mediterranean. They all adhere to the under side of stones, shells, *etc.* A vertical section of the simplest species, *Plakina monolopha*, is given in Pl. XXV, Fig. 7. There is a continuous basal cavity crossed by strands of tissue, in which lie developing eggs. From the cavity run vertical efferent canals (*cf. c.*), which are simple or very slightly branched, and into which open the

flagellated chambers. The afferent canals (*af. c.*) are spacious cavities opening on the surface by wide mouths. The periphery of the sponge forms a continuous rounded rim (the "ringwall," *r. w.*), and the oscula, one or several, are situated here. The surface of the sponge inside the ringwall is divided up into low rounded elevations, caused by the upper ends of the efferent canals, between which lie the wide apertures leading into the afferent canals. Schulze was fortunately able to observe the main features in the development of this interesting form. There is a solid swimming larva which settles down, forming a flat circular mass. A central cavity appears in the mass, the lining cells becoming columnar, and the sponge is thus transformed into a flat three-layered sac, Pl. XXV, Fig. 8, the three layers being respectively ectoderm, mesoderm, and entoderm. The flagellated chambers appear in a single layer round the central cavity, into which they open. They are very probably formed as diverticula of this cavity. Schulze did not follow the development further, but a comparison of the adult with the sac-like young form makes it pretty certain that the young form undergoes a process of folding which gives rise to the efferent and afferent canals of the adult; or in other words the efferent canals arise as vertical evaginations of the sac-like stage. The afferent canals are consequently to be regarded as lined with ectoderm.

A vertical section of the second species, *Plakina dilopha*, is shown in Pl. XXV, Fig. 9, and of the third species, *Plakina trilopha*, in Pl. XXV, Fig. 10. The oscula in these species are not situated at the periphery as in *Plakina monolopha*, but at some distance internal to it; and in them the efferent canals do not form projections on the surface as in the first species. On comparing the canal systems in Figs. 7 and 9 it is seen that *Plakina dilopha* has probably been derived from *Plakina monolopha* by an increase in the thickness of the mesoderm lying beneath the surface of the sponge. The wide afferent canals of *Plakina monolopha* become transformed into the narrow efferent canals of *Plakina dilopha*. In other respects there has been no great change. (Schulze, pp. 438 and 439.)

Plakina trilopha goes a step farther in the direction of complexity than does *Plakina dilopha*. It has probably been derived from the latter species (compare Figs. 9 and 10) by the appearance of secondary folds in the radial efferent tubes; by a transformation of the basal cavity into a system of lacunae, owing to the increase in number of the connecting strands of tissue between the basal layer and the part of the sponge containing the flagellated chambers; and by a complication in the afferent canals in consequence of which they do not open each by a single aperture but by a number of small apertures, the surface pores (*s. p.*).

Schulze's conclusion that these species all lie in one line of descent, that is that the second has been derived from the first, and the third from the second, receives as much support from a study of the spicules, as of the canal system. But on this head, reference will have to be made to the original paper.

From comparative anatomy we conclude that the phylogeny of the sponges is something as follows: The *Olynthus* is the common ancestor of the group. The outgrowth of radial tubes gave rise to the *Sycon* type. The growth of the mesoderm and development of new endodermic diverticula, coupled with the metamorphosis of radial tubes into flagellated chambers, produced the *Leucons*. The non-calcareous sponges have been derived from types with a canal system more or less like that of the *Leucons*. And the conclusion with regard to the germ layers is that the efferent system is entirely endodermic, and the afferent system entirely ectodermic.

Embryological Evidence. — Let us see now how far the known facts of development support the above conclusions. The evidence from the calcareous sponges (*Sycandra* passes through *Olynthus* stage) has already been given. Several of the non-calcareous sponges (*Oscarella lobularis*, *Reniera filigrana*, *Chalinula fertilis*, *Plakina monolopha*) run through a stage known as the *Rhagon* (Sollas), which it is permissible to regard as the ontogenetic representative of the *Sycon* type. The *rhagon* of *Oscarella* (Heider 9) is shown in Pl. XXV, Fig. 11. Regarding it, as seems best, as equivalent to the *Sycon* type, it will be noticed that the radial tubes of the

Sycon are coenogenetically replaced by flagellated chambers. The rhagon of *Oscarella* is formed as an invaginate gastrula, which attaches mouth down. The gastrula mouth closes, and the osculum is a new formation. The flagellated chambers arise as true diverticula of the central cavity. The adult *Oscarella*, the canal system of which is not far removed from that of *Plakina monolopha*, is very probably formed from the rhagon, by the development in the latter of a number of simple diverticula from the central cavity. These diverticula are the efferent canals into which open the flagellated chambers. The ectodermic spaces between the efferent diverticula become the afferent canals. The adult *Oscarella*, like *Plakina monolopha*, is directly comparable with a simple *Leucon*. The development of *Oscarella* in large measure confirms the conclusions drawn from comparative anatomy, and may therefore be considered as phylogenetic.

The development of *Plakina monolopha* (Schulze) has already been described. The sac with its single layer of flagellated chambers opening into it, is a rhagon, and may be taken as representing the Sycon stage. The adult *Plakina* itself is the *Leucon* stage.

In *Reniera filigrana* (Marshall 18) there is a solid swimming larva, which after attaching acquires a central cavity with an apical osculum. The flagellated chambers arise as diverticula from this cavity. Thus in this sponge also there is a rhagon stage. But in one matter we strike upon a coenogenetic modification. The afferent canals, instead of being ontogenetically formed from the ectoderm, as they seem to have been phylogenetically, are really formed from endodermic diverticula, which grow outwards, meeting the surface epithelium.

In *Chalinula fertilis* (Keller 10) there is also a solid larva in which a central cavity is hollowed out. But in this sponge the flagellated chambers of the rhagon stage do not arise as endodermic diverticula, but are formed independently from solid groups of mesoderm cells. This origin of the flagellated chambers must be regarded as coenogenetic. The fact that the mesoderm may take upon itself the function of forming organs ordinarily formed by the entoderm would seem to

indicate that the two layers are of much the same nature. This essential similarity between the two layers has always been maintained by Metschnikoff, not only on the ground of development, but for physiological reasons as well. Thus in young *Spongillas* when the water became bad, he has witnessed the entire disappearance of the flagellated chambers, the sponge then consisting of ectoderm and mesoderm alone. With a fresh supply of water the chambers reappeared (12, p. 375). Again, after feeding carmine in an excessive amount to *Halisarca pontica*, he found that the canals and chambers entirely disappeared, the whole body of the sponge inside the ectoderm consisting merely of a mass of amoeboid cells full of carmine (*ibid.*, p. 372). The development of the afferent system in *Chalinula* was not worked out with certainty.

The embryology of the preceding sponges, in which a rhagon type is developed, agrees pretty well with our general notions of sponge phylogeny. But there are other sponges, the development of which has been so excessively modified as no longer to be of any use as finger-posts to phylogeny, but which afford an excellent field for the study of what may be called the methods of coenogeny. In *Halisarca Dujardinii* (Metschnikoff 12), for instance, there is a solid larva in which the canals appear as so many separate lacunae surrounded by parenchyma (mes-entoderm) cells. The canals only subsequently acquire a connection with each other.

In *Esperia* (Maas 16), the subdermal spaces, canals, and chambers arise separately as lacunae in the parenchyma. The chambers are formed from aggregations of small cells (which Maas believes, on what seems to me insufficient evidence, to be ectoderm cells of the larva that have migrated into the interior). The efferent canals, Maas thinks, are formed from similar cells.

In *Esperia*, according to Yves Delage (36), the chambers arise by division of special mesoderm cells. The epithelium of the canals comes from the larval ectoderm, which migrates into the interior. In *Spongilla*, according to the same author, the ectoderm cells of the larva are *engulfed by mesoderm cells*, and then become the lining cells of the flagellated chambers!

In young *Stellettas* (Sollas 28, pp. xvi-xvii) the subdermal cavities seem to arise as lacunae in the parenchyma. And in the external buds of *Tethya maza*, Selenka (29) believes the subdermal cavities have a similar origin.

In *Spongilla*, according to Götte (6), the subdermal cavities and canals are formed as independent lacunae in the parenchyma, and the flagellated chambers are formed from groups of cells, each group (and chamber) being produced by the budding of a single large mesoderm cell. This account of the development of *Spongilla* is contradicted by Maas (14) who brings *Spongilla* in line with those forms having a rhagon. Maas describes in the larva a single central cavity from which the chambers arise as diverticula, the central cavity persisting in a modified shape as the efferent system of canals. The subdermal spaces arise as ectodermal invaginations, from which the afferent canals are formed as ingrowths. Thus according to Maas in the ontogeny of *Spongilla*, the whole afferent system is formed from the ectoderm and the whole efferent system from the endoderm. Ganin's earlier account (7) likewise makes the chambers originate as diverticula from a main endodermic cavity.

In the metamorphosis of a larva, which probably belongs to *Myxilla*, Vosmaer (34) finds the subdermal cavities begin as fissures which gradually become wider, and the canals and chambers likewise appear as intercellular spaces.

Finally, in the gemmule development of *Esperella* and *Tedania* I find that subdermal cavities, both sorts of canals, and the flagellated chambers, all arise as independent lacunae in the parenchyma.

Accepting as ancestral the development (*i.e.* later development or metamorphosis) of *Oscarella* and *Plakina monolopha*, the various coenogenetic modifications which appear in other sponges may be classified as follows:—

1. The efferent canal system, instead of arising as a single cavity which throws out diverticula, may be formed as so many distinct cavities which subsequently unite (*Esperella*, *Tedania*, *Esperia* [Maas], *Halisarca Dujardinii*, *Myxilla*).

2. The flagellated chambers, instead of arising as endodermic

diverticula, may be formed from groups of mesoderm cells (Esperella, Tedania, Chalinula fertilis, Myxilla).

3. The afferent canals, including the subdermal cavities, instead of being formed as invaginations from the ectoderm, arise as lacunae in the mes-entoderm (Esperella, Tedania, Esperia, Stelletta, Myxilla). In Reniera filigrana (Marshall) they are formed as entodermic diverticula.

The coenogenetic development of the flagellated chambers and efferent canals suggests, as I have said, an essential similarity of nature in the so-called entoderm and mesoderm of sponges. This belief, so long upheld by Metschnikoff, derives some of its strongest support from this author's physiological investigations (see *ante*, p. 359), as well as from the fact, first emphasized by Metschnikoff and Barrois, that in the most common sponge larva (the solid larva) mesoderm and entoderm form a single indivisible layer.

And likewise the development of the afferent system of canals, in some sponges from the ectoderm, in others from the mes-entoderm, may possibly be taken as meaning that even these two primary layers (the outer and the inner) are not distinctly differentiated from each other in the sponges; or, in other words, that the mes-entoderm is still enough like the ectoderm to form organs ordinarily produced by the latter layer.

There is another (hypothetical) way of explaining these phenomena, which consists in supposing that ectoderm cells of the larva migrate into the interior, and, although indistinguishable from the surrounding mes-entoderm cells, alone take part in forming the afferent canals. Similarly we may suppose that in the solid mass which constitutes the parenchyma of Esperella there are two radically distinct classes of cells, one of which is potentially gifted with the power of forming efferent canals and flagellated chambers, while the other has not this power and must remain as amoeboid mesoderm. But this is pure hypothesis.

The result of this critical examination seems to be that the Olynthus must be regarded as the ancestor of sponges (Haeckel, Kalk-spongien), and that the entoderm and mesoderm are not

sharply differentiated from each other as they are in the higher animals (Metschnikoff, *Spong. Studien*, p. 378).

Origin of the Olynthus.—The prevalence of the solid larva in sponges and hydromedusae, coupled with the widespread presence of intracellular digestion in the lowest metazoa, led Metschnikoff years ago to the belief that the solid larva represents the ancestral form of the metazoa, while the gastrula is a coenogenetic modification (12, 13). To my own mind, all the facts that we know indicate this conclusion to be well founded. This hypothetical ancestral form was named *Parenchymella* (changed later to *Phagocytella*). I may be permitted to recall its leading features as deduced by Metschnikoff. The animal consisted of an outer layer of flagellated cells and an inner mass of amoeboid cells. The digestion was intracellular, the food being taken in through openings scattered over the surface. A central cavity having a special opening to the exterior was a later acquisition, the opening being in all probability one of the small apertures especially enlarged. This solid ancestor of the metazoa, Metschnikoff derives from colonial forms like *Protospongia*. Barrois (1), as early as 1876, stated his belief that the ancestor of sponges was a solid animal composed of two layers, the outer representing the ectoderm, the inner mass representing a parenchyma, from which have developed the ectoderm and mesoderm of higher animals (p. 78).

According to this view, the early development of *Plakina* (or *Reniera*, *Chalinula*, etc.) gives the first chapters in the history of the group of sponges more faithfully than does a form like *Oscarella* or *Sycandra*. In the former sponges, it will be remembered, there is a solid larva hollowed out to form a three-layered sac, which then breaks open to the exterior, forming the osculum. In the latter there is an invaginate gastrula which settles mouth downwards, the gastrula mouth subsequently closing and the osculum appearing as a perforation at the upper end of the sac. In these forms, *Oscarella* and *Sycandra*, we have to suppose that the *Parenchymella* stage is skipped, the central cavity (which properly belongs to the *Olynthus* stage) being precociously developed coincidently with

the immigration of the entoderm. The blastopore of the sponge gastrula on this view does not represent a primitive organ (Urmund), but merely comes into existence owing to the highly modified method of forming the entoderm. We do not, therefore, have to construe the Oscarella development (with Heider and Sollas) as meaning that a gastraea ancestor settled mouth downwards, and that the mouth gradually became functionless, finally closing up, while a new series of openings, pores and oscula, was established.

The only remaining point I wish to speak of is the relation of the sponges to the coelenterates. That the two groups have had a common ancestor in the *Parenchymella* is highly probable, but the similarity between the *Olynthus* and the simplest coelenterates inclines one to go further and, at any rate, homologize the paragastric cavity of the former with the gastric cavity of the latter. This, of course, is done by authors like Sollas, who derive both groups from a gastrula-like ancestor. Whether the osculum of the *Olynthus* is also homologous with the coelenterate mouth, as Haeckel originally held, is a question which needs for its answer more facts relating to the actual use to which the osculum is put in the simplest sponges. Sollas and Heider urge against the homology, the fact that the coelenterate larva attaches by the pole opposite the blastopore, while in the sponge larva the blastopore is at the pole of attachment. But this I cannot regard as a very strong argument, for I do not believe that the opening into the gastrula cavity represents a primitive organ (mouth of an ancestor). And if it does not, but is merely an incidental product of a particular mode of endoderm-formation, it becomes evident that the position of the blastopore at opposite poles in sponge and coelenterate larvae has no bearing on the question of homology between mouth and osculum.

It is, moreover, doubtful if any such sweeping distinction can be drawn between the larvae of the two groups, for it is a question whether any sponge larva has a particular pole by which it must attach. Even in *Sycandra*, Schulze records (25, p. 274) that exceptional cases occur which cannot be regarded as pathological, in which fixation takes place not by

the gastrula mouth, but on the side. Fixation may also be delayed until the gastrula mouth has closed and spicules have begun to appear, in which case it is not stated by what part the larva attaches. In the solid larvae of silicious sponges the variation is much greater. Such larvae attach in some cases by the posterior pole, in others by the anterior pole, and yet in others on the side. All these variations may occur in larvae of the same species, for instance Maas records (16) that in *Esperia* he observed fifteen individuals attach by the posterior pole, seventy by the anterior pole, and five or six on the side. It thus appears that in the larvae of silicious sponges at any rate there is no constant point of attachment.

VI. REMARKS ON THE GEMMULE DEVELOPMENT OF SPONGES.

1. *Asexual Development in General of the Sponges.*

The asexual method of development exhibits itself in sponges in a variety of ways. Besides the simple coelenterate-like process of budding which leads either to the production of new individuals or to the formation of more or less clearly marked colonies, and which is seen at its simplest in the calcareous sponges, the following instances of non-sexual reproduction may be called to the mind of the reader.

In *Oscarella*, Schulze (27) found that hollow outgrowths were constricted off from the surface of the sponge, which led a free-swimming life for several days, ultimately sinking to the bottom and developing each into a new sponge. The outgrowth contained a diverticulum from the canal system of the mother, and the wall of the outgrowth agreed in structure with the wall of the parent sponge, *i.e.* it contained flagellated chambers with the short afferent and efferent canals. The method of bud formation here employed seems to be fundamentally the same as that exhibited in the calcareous sponges and the coelenterates.

The propagation of sponges by cuttings may be mentioned in this connection. The experiments of Oscar Schmidt and those of the U. S. Fish Commission (made on the Florida

coast) have demonstrated that small pieces or cuttings of the commercial sponge have the ability to reproduce the entire organism. Cuttings from *Tedania*, which were suspended from mangrove roots in one of the "sounds" of Green Turtle Cay, grew very perceptibly in a month.

In *Tethya* and *Tetilla* (Selenka 29, Deszö 3, 4) external buds are produced in a curious way. The buds consist of a solid mass of cells, and are formed in the peripheral region of the mother beneath the skin. As they mature they are gradually pushed out of the body along the spicules of the mother, until their only connection with the parent is through a slender stalk made up chiefly of these spicules. The bud then drops off. Deszö's account of the early formation of these structures is extremely interesting, although his recorded facts scarcely seem to warrant his inferences. According to Deszö, the bud or gemmule is derived from a single cell, which undergoes a segmentation, and growing all the while gives rise to a solid morula. By the time the original cell has divided into four, a differentiation of "germ layers" takes place: one of the four cells constitutes the entoderm, the remaining three the ectoderm. The ectoderm then grows entirely round the entoderm cell. Cell multiplication continues, the primary entoderm cell producing a solid mass of entoderm, surrounded by a single layer of ectoderm cells. The latter layer then splits off from its inner surface a layer of mesoderm, and itself gives rise to the external epithelium of the mature bud and to a stratum of tissue just beneath the epithelium, in which small asters (spicules) are developed. Deszö's interpretation of certain cells as constituting distinct germ layers, is not very strongly supported by his figures. Vosmaer's criticism in regard to this point may be given: "Es ist wohl klar dass für die Deutungen der Zellen, wie sie Deszö vornimmt, kein Grund vorliegt" (Bronn's Class. und Ordnung, p. 427). Deszö points out the importance, from a biological standpoint, of the discovery of germ layers in a non-sexually produced embryo, and calls to mind a similar discovery by Oscar Schmidt in the developing buds of *Loxosoma*. Schmidt's account of the development of the *Loxosoma* buds (23, 24),

however, has not been confirmed by later investigators. On the contrary Seeliger finds that the bud is not derived from a single cell, but is formed in a very different way. According to Seeliger (30, 31) the bud, both in *Loxosoma* and *Pedicellina*, is formed as a papilla of the body wall (or stolon, in the case of *Pedicellina*), an invagination at the end of the papilla giving rise to the atrium and alimentary tract. The mesoderm of the bud is derived from the mesoderm of the parent, the ectoderm of the bud is derived from the ectoderm of the parent; and the only new formation is the entoderm, which is produced by an *invagination of the adult ectoderm*. Seeliger's account destroys the possibility of drawing a parallel between sponge gemmules, which develop germ layers, and the buds of *Loxosoma*.

The internal buds or gemmules of the fresh-water sponges have been known since the time of Linnæus, but their precise origin is still open to discussion.

The ripe gemmule consists of a solid mass of polygonal cells, full of yolk, surrounded by a complex capsule. The capsule is perforated by an opening (hilum), through which in the spring the cellular mass creeps out, developing into a new sponge. The capsule is composed of an inner and outer cuticular layer, between which is a layer containing skeletal elements (amphidisks or other spicules). According to Götte (6), all the cells in a particular region of the body of the parent sponge, not only those of the mesoderm, but those of the flagellated chambers and canals as well, become transformed into a mass of yolk-containing cells, which constitutes the gemmule. According to Marshall (19), however, the gemmule is formed exclusively from an aggregation of mesoderm cells. In whichever way formed, the young gemmule becomes differentiated into two layers, an inner mass of larger cells full of yolk, and a peripheral layer of cells (Götte). According to Götte, the peripheral layer of cells secretes the inner and outer cuticle, and gives rise to the amphidisks. According to Wierzejski (cited from Vosmaer 33, p. 429), the peripheral layer assumes the character of a columnar epithelium. Between it and the central mass appears the inner cuticular layer. The spicules and amphidisks are formed entirely outside the gem-

mule, in the parenchyma of the mother sponge, and only after formation do they get into the peripheral layer of the gemmule. The cells of the latter layer, however, secrete the outer cuticle, and subsequently entirely disappear.

Gemmules fundamentally like those of *Spongilla* have been found in certain marine sponges by E. Topsent (32). The sponges in which the gemmules were observed are *Chalina oculata*, *Chalina gracilenta* (I have seen them myself in *Chalina arbuscula*, Verrill, during the summer at Woods Holl), *Cliona vastifica*, *Suberites ficus*. The gemmules consist of a mass of cells surrounded by an envelope of horny matter (keratode), the protoplasm of the cells being full of highly refractive granules (presumably yolk). An earlier notice (1880) of the existence of such gemmules in marine sponges is contained in Claus's *Grundzüge*, Bd. I, p. 214: "Auch bei den Meereschwämmen ist die Vermehrung durch Gemmulae verbreitet. Dieselben entstehen unter gewissen Bedingungen als kleine von einer Haut umschlossene Kügelchen, deren Inhalt im Wesentlichen aus Schwammzellen und Nadeln gebildet ist und nach längerer oder kürzerer Zeit der Ruhe nach Zerreißen der Haut austritt."

In *Craniella* are found embryos which Vosmaer interprets as gemmules (Bronn's *Class. und Ord.*, p. 428). Sollas has, however, seen the same structures and regards them as egg embryos (28, pp. 33-39).

Oscar Schmidt (22) stated it as his opinion that there was no true segmentation in the eggs of horny and silicious sponges, but that the egg very early lost its cellular character. It seems probable that Schmidt had seen cases of gemmule development, more or less like the development of *Esperella* and *Tedania*, as described by myself.

The ciliated larvae of species of *Esperia* (*Esperella*) have been repeatedly seen and studied (Metschnikoff 11, Carter 2, Schmidt 22, Maas 16, Yves Delage 36). It has been assumed in all cases that the larva observed was an egg larva, and of course this may have been true. The close resemblance, between the larvae observed by Maas and myself, suggests, however, that the former larvae were, like mine, gemmule larvae.

2. *Comparison between the Egg Larva and Gemmule Larva of Silicious Sponges.*

Resemblance of the two kinds of larvae. — A comparison of the gemmule larvae I have described, with the egg larvae of silicious sponges, reveals the fact that the two are similar in essential respects. These essential points are the presence and character of the germ layers, and the peculiar differentiation of one pole. The similarity between the two sorts of larvae will be seen after a brief survey of what is known concerning the egg larvae of silicious sponges.

The larva of *Isodyctia* (Barrois 1) is a solid oval larva (parenchymella). Except at the posterior pole it is everywhere covered by a layer of columnar ciliated cells, the ectoderm. At the posterior pole, according to Barrois, the ectoderm is absent and the inner mass (mes-entoderm) is laid bare to the exterior. In this larva as in others in which it has been claimed that the entoderm is exposed to the exterior through a break in the ectoderm, recent investigations (especially Maas's study of the flattening of the columnar epithelium in *Spongilla*, and the facts recorded in the descriptive part of the present paper) make it probable that the mes-entoderm is really not laid bare but is covered by a layer of flat ectoderm cells. The unciliated posterior pole of the *Isodyctia* larva is made further noticeable by a deposition of red pigment in its cells, and the cilia immediately surrounding it are unusually long, forming a conspicuous circle. In this larva the posterior pole ("calotte") is at no time ciliated nor, as I understand the author, is it ever covered (not even before birth) with columnar ectoderm. The resemblance between this larva and the gemmule larva of *Esperella* is, to say the least, striking.

The larva of *Desmacidon* (Barrois 1) is very like that of *Isodyctia*. As in the latter sponge, the columnar ciliated ectoderm is lacking at the posterior pole which is further distinguished by its pigment and by a circle of long cilia immediately surrounding it. Unlike *Isodyctia*, the larva of *Desmacidon* when set free is ciliated all over. But pigment

accumulates at its posterior pole, and the cilia and "son revêtement cellulaire" disappears (this, as I have said, is probably to be interpreted as meaning that the columnar ciliated ectoderm of this pole becomes transformed into flat unciliated cells). In this last detail the *Desmacidon* larva is more like *Tedania* than *Esperella*. It will be remembered that in the *Tedania* embryo all the ectoderm cells become columnar, those at the posterior pole subsequently flattening out. In *Desmacidon* and *Isodyctia* both, the ectoderm is subsequently "broken through" at the anterior pole also. But this, it would seem, is only the first step in the general flattening of the ectoderm.

The larva of *Reniera filigrana* (Marshall 18) is a solid oval larva with a pigmented pole, and covered at first uniformly with columnar ciliated ectoderm. Unlike the two preceding sponges, the pigmented pole is the anterior. The ectoderm "bursts" at the pigmented pole, and the mes-entoderm is laid bare. Subsequently the ectoderm "bursts" at the opposite pole, and at about the time of fixation the whole ectoderm flattens.

In *Chalinula fertilis* (Keller 10) there is a solid larva essentially like the gemmule larva of *Esperella* and *Tedania*, in that the columnar ciliated ectoderm is absent at the posterior pole. According to Keller, this larva is derived from an epibolic gastrula, and the cells occupying the posterior pole are a part of the mes-entoderm, which is here from the start exposed to the exterior. My observations on the way in which this pole is formed in *Esperella* and *Tedania*, make it probable, I think, that the surface cells in this region of the *Chalinula* larva are ectodermic. Indeed, for a short time after birth the posterior pole is ciliated, but the cilia are, however, soon lost. Believing, as I have said, that the inner mass of cells is from the beginning exposed to the exterior at the posterior pole of the embryo, Keller naturally regards this region as a blastopore.

Vosmaer (34) describes the development of a larva which probably belongs to the genus *Myxilla*. The larva is solid and is covered with a cylindrical epithelium. A portion of the surface loses its cilia, the cells becoming cubical. Attachment

takes place in the region of the cubical cells, and the larval epithelium is not lost, but is modified cell by cell.

The larva of *Amorphina* (Schmidt, 22) is a solid larva ciliated all over. The cilia are lost at the posterior end. In the same paper, Schmidt describes the larva of a species of *Esperia*. The larva is solid and is ciliated all over. The cilia are lost at one pole, the spicules collecting at this pole. The larva of *Reniera* (Schmidt, *l. r.*) is a solid ciliated form with a deeply pigmented pole. The cilia on the pigmented pole are lost. There are other observations by Metschnikoff (11) and Carter (2) to the effect that the ectoderm is absent at the posterior pole of the larvae of silicious sponges. In these cases, as in the case of the *Myxilla* larva described by Vosmaer, it remains doubtful until the early development is known, whether the larva is really an egg-larva.

It will be seen that the larvae of the above-mentioned silicious sponges agree in fundamental respects. They all consist of two germ layers: an inner parenchymatous mass (mes-entoderm) and an outer layer of columnar ciliated cells (ectoderm). At one pole, usually the posterior, the ectoderm is apparently absent, the appearance being probably due to the fact that at this pole it is composed of flat unciliated cells. Like the egg larvae, the gemmule larvae (of *Esperella* and *Tedania*) consist of two germ layers, an inner parenchymatous mass (mes-entoderm) and an outer layer of columnar ciliated cells (ectoderm), the columnar ciliated cells giving place to flat unciliated cells at the posterior pole which is thus differentiated. It is plain that the two sorts of larvae agree in essential structure.

There are other silicious sponges, *Spongilla* (6, 14), the tetractinellid form *Plakina monolopha* (26), and *Tedanione* (see *ante*, p. 345), in which the larva has not the peculiar differentiation of one of the poles which is seen in the above-mentioned forms. But this differentiation is so common that it may fairly be considered as typical of a large though ill-defined group of sponges.

Cause of the Resemblance.—Accepting as a fact the resemblance between the egg and gemmule larvae in the possession

of germ layers and the differentiation of one of the poles, we must now put the question as to the cause of the resemblance.

We have for long been accustomed to regard the two primary germ layers of an embryo as representing the primitive metazoan organs, *i.e.* the outer (nervous) and inner (digestive) layers of a simple two-layered form. It is possible that this view is not an entirely correct one, and that many so-called germ layers are not the ontogenetic representatives of the layers of the metazoan ancestor. And the occurrence of germ layers in an asexually produced embryo may possibly be interpreted as favoring the latter belief. It seems to me, however, that, while it is perhaps permissible to *suspect* the doctrine that the primary germ layers are homologous (I refer of course to the general homology maintained by Balfour in his *Comp. Embr.*, Vol. 2, p. 286) throughout the metazoa, we are not at present in a position which would warrant our giving up the doctrine. Certain it is that some form of two-layered embryo is found in every group, and that the various forms may be considered as modifications of a type; and, to my mind, the best explanation of these facts is still the old one, that the germ layers are inheritances from a far distant two-layered ancestor.

Accepting the premise that germ layers are not independently acquired, but are inheritances from a common stock, we reach the conclusion that an asexually developed embryo (sponge gemmule) can reproduce features of a far distant ancestor (germ layers).

Coming now to the second point of resemblance (differentiation of a pole) between the egg and gemmule larvae of silicious sponges, we have first to ask ourselves, what is the meaning of this curious differentiation of one of the poles in the egg larva itself. This question I am quite unable to answer. Barrois (1) and more recently Keller (10) have regarded the unciliated pole as a blastopore, thus making it possible to compare the larva of silicious sponges with the amphiblastula of calcareous sponges. The basis on which their view rests is, that the endoderm at the pole in question is exposed to the exterior, and this, it is pretty certain, is not the case. The differentiation of the pole can have no such deep-seated morphological

significance as is advocated in this theory. It is probably an adaptive feature acquired within the group of silicious sponges. Now whether the gemmule larva has independently acquired this adaptation, is open to discussion. I am inclined to believe that it exhibits the feature in question, for the same reason that it develops germ layers: both features come to it as inheritances, the latter from a far distant ancestor, the former from a comparatively near one, both features being of actual physiological use to the larva.

To repeat, the conclusion I reach in regard to the marked resemblance between the egg larva and gemmule larva of silicious sponges is, that it is one not due to independent adaptation to similar circumstances, but to inheritance from a common source. What I believe I have found is, a bud embryo exhibiting ancestral traits. To illustrate by means of an imaginary example: suppose the bud of a simple ascidian, instead of developing directly into a new ascidian, first developed into an ascidian tadpole, with its notochord, nervous system, *etc.*, we should then have, I take it, a parallel case to the gemmule development of sponges. Only, in the imaginary case there could be no doubt of the larval features being inheritances, while in the case at hand I am free to admit that this view could be disputed.

The exhibition of ancestral traits in a bud embryo is perhaps a very rare phenomenon. The supposed occurrence of this phenomenon in *Loxosoma* has been shown to be without foundation, and Deszö's claim that it does occur in the development of *Tethya* buds cannot, in view of the insufficient evidence, be admitted. (Moreover, if Deszö's statement that the *Tethya* bud is derived from a single cell, be a fact, such a cell could properly be regarded as an undeveloped germ cell, and the "budding" of *Tethya* would then be a process analogous to the "sporogonie" discovered by Metschnikoff in *Cunina proboscidea* (38), or to the paedogenesis of the *Cecidomyia* larva, and therefore not a case of asexual reproduction.) In fact but a single instance of this phenomenon, as far as I know, has been recorded for the animal kingdom, previously to the appearance of these observations. The case referred to

is the remarkable development of the hydromedusa, *Epenthesis McCradyi*, described by Brooks (39). The novel development of this jelly-fish is thus sketched in the opening paragraph of Professor Brooks's paper: "In June, 1889, I found at Nassau, N. P., in the Bahama Islands, a few specimens of a hydromedusa belonging to the family Eucopidae (Haeckel), bearing upon each one of its four reproductive organs a number of hydroid blastostyles from which young medusae are produced by budding; a method of reproduction which has no exact parallel among the hydroids nor, as far as I am aware, anywhere else in the animal kingdom; for the reproduction, by a medusa, of blastostyles which are morphologically equivalent to hydras, is a reversion, through asexual reproduction, to a past larval stage; a phenomenon which is thoroughly anomalous and exceptional."

While Brooks regards the production of blastostyles on the medusa as a case of asexual reproduction, he finds they are not produced as simple buds. The ectoderm of the blastostyle is continuous with the ectoderm of the medusa, and arises as a bud-like outgrowth from the latter. The endoderm of the blastostyle has, however, no connection with the endoderm of the medusa, but is rooted in the mass of germ cells composing the reproductive organ of the latter. "These germ cells give rise to the endoderm of the blastostyle by a process of specialization which is very similar to what Metschnikoff has described in *Cunina* and has termed *sporogenesis*." The formation of blastostyles in *Epenthesis* is thus a composite method of reproduction, a part of the blastostyle being formed by budding, and a part by the development of rudimentary germ cells. Professor Brooks's opinion of this interesting development had best be given in his own words: "It is probable that *Epenthesis* is also an example of sporogenesis, and that the endodermal tube is derived from a single cell by segmentation, but this is certainly not true of the ectoderm of the blastostyle, and if we have sporogenesis at all in *Epenthesis*, we have it in combination with budding."

At the root of Weismann's theory of inheritance lies the supposed essential difference between somatic and germ cells.

In a little paper (35) embodying the main results of the present one, I endeavored to ascertain in terms of Weismannism the precise nature of the cells which combine to form the sponge gemmule, and arrived at the conclusion "that the gemmule cell, according to this view (Weismann's) must be regarded as a true germ cell, in which all the germ plasm remains undifferentiated, *viz.* in which none of it is transformed into ovogenetic plasm. Further, the gemmule cell pursues the parthenogenetic course of development—it keeps all its germ plasm" (p. 579). But at bottom it does not seem to me that a case of this kind, in which there is essential similarity between the products of a developing bud and a developing egg, tends to strengthen Weismann's fundamental proposition that germ cells and somatic cells are radically different.

Appendix.—I am fortunately able, some months after the completion of the present paper, to notice the remarkable memoir on the development of sponges, which M. Yves Delage has recently published.¹ In this memoir Delage describes in detail the post-larval development of *Spongilla*, *Reniera*, *Aplysilla*, and *Esperella sordida*. The essential features of development were found to be the same in all. I will briefly review his account of the *Esperella* development, and will then comment on certain points in which the account agrees or differs with mine.

In the ciliated larva Delage distinguishes four classes of cells each of which is destined to form a particular part of the adult body. There is a covering layer of ciliated cells, wanting at the posterior pole. Scattered about between the basal portions of these cells is a discontinuous layer of cells called by the author *epidermic*. At the posterior pole these lie at the surface, forming a nearly complete layer (in similar larva of *Reniera* they form, according to Delage, a complete layer). The remaining inner mass is composed of amoeboid and *intermediary* cells, the latter immobile and of a rather negative character.

The ciliated cells absorb their flagella and migrate into the interior, ultimately becoming the lining cells of the flagellated

¹ Embryogénie des Éponges. Archives de Zoologie Expérimentale et Générale. Année 1892. No. 3.

chambers. Simultaneously the epidermic elements come to the surface and fuse with one another to form a complete membrane, the definitive epidermis. The amoeboid cells become the wandering cells of the adult mesoderm, while a part of the intermediary cells form the epithelium of the canals, the rest becoming the stationary elements of the mesoderm. These conclusions differ, it will be seen, in some important respects from those presented by the author in his preliminary notes (*Comptes Rendus* 1890, 1891), cited *ante*, p. 317, 359.

Formation of epidermis.—In believing that the cells which cover the posterior pole form a different part of the adult body from the rest of the covering cells of the larva, I think Delage is wrong. That no such distinction exists between these two sets of the superficial cells of the larva, is made probable at the very beginning where it is seen that the young embryo is covered with a continuous layer of similar cells (columnar in *Tedania*, 35, p. 576, and *ante*, p. 330), which subsequently differentiate into the ciliated cells and the flattened ectoderm of the posterior pole. Delage like myself is unable to offer a satisfactory explanation of the peculiar character of this pole. He does put forth the suggestion that it is due to a rupture in the covering of ciliated cells, produced at a point of weakness by the growth of the inner mass. But the observation I have just cited upsets such an explanation.

The immigration into the interior of a part of the ciliated cells, I am prepared to believe in, some of my own observations suggesting, though by no means proving, the occurrence of such a phenomenon (*ante*, p. 299). On the other hand I am sceptical as to the existence of Delage's layer of epidermic cells, not having found any such layer in the larvae I have studied. I regret that my observations on the actual transformation of the ciliated cells of the larva into the flattened epidermis of the adult are so meagre, but such as they are they are in harmony with the views of those writers (*ante*, p. 301) who claim to have seen such a transformation, and not with the views of Delage. It may be mentioned that Delage finds the formation of the definitive epidermis to begin at the anterior pole and gradually progress towards the posterior pole

of the larva. In *Esperella fibrexilis* I have found the process, interpreted so differently, to take place in the opposite direction.

Marginal Membrane.—A marginal membrane, essentially like the ectodermal membrane I have described surrounding the young *Esperella* and *Tedania*, is formed in all the sponges studied by Delage. The author's account of the manner in which the membrane is formed differs, however, from mine. In *Spongilla* and *Esperella sordida* Delage describes the marginal ectodermic cells of the just attached sponge as creeping outwards in an amoeboid fashion and so forming a considerable membrane, at the edge of which the cells remain amoeboid (Pls. XIV, XV). As my figures show I have never found the marginal ectodermic cells amoeboid. On the contrary I have found the ectoderm (epidermis), as it extends out to form the membrane in question, retaining a continuous edge, which could not be the case if the individual cells of the margin threw out processes (*ante*, pp. 303 and 335, and especially Pls. XXI and XXII). The condition, at least the later condition, of the membrane in *Aplysilla*, as described by Delage, accords better with my observations than does his account of the membrane in *Spongilla* and *Esperella*. In *Aplysilla* the marginal epidermic cells at first throw out amoeboid processes, but later assume regular shapes, and arrange themselves alongside one another in such a way as to give to the membrane an even continuous edge.

Flagellated Chambers.—Several of the stages in the formation of the chambers that M. Delage has found, are quite like such as I have seen, but the whole process is construed very differently. Delage's account is as follows: "The ciliated cells after their migration into the interior are seized upon and engulfed, amoeba-fashion, by the amoeboid cells. Complete fusion takes place between the bodies of the absorbed cells and that of the amoeboid, but the nuclei of the former remain distinct and arrange themselves round the much larger nucleus of the latter. In this way are formed the multinucleate cells which have been interpreted so differently by previous observers. In *Spongilla* all the ciliated cells are absorbed by

the amoeboids. In *Esperella* and the other sponges only a portion are so absorbed, while the remainder throw out processes and unite with one another and the now multinucleate amoeboids, to form a syncytial net-work. In the development of a chamber several of the multinucleate masses approach one another and form a continuous wall round a central space. The space becomes the cavity of the chamber, round which the nuclei of the absorbed ciliated cells arrange themselves in a regular fashion, while the nucleus of the amoeboid surrounded by protoplasm escapes from the periphery of the chamber anlage, and becomes a wandering cell of the mesoderm. The ciliated cells not associated with the amoeboids, but which are merely part of the syncytium, unite in the same manner and form chambers."

The multinucleate formative cells I have described evidently correspond to Delage's multinucleate amoeboids. But while Delage agrees with Götte and myself (35, and *ante*) in regarding the smaller peripheral bodies as nuclei, he differs completely in his explanation of their origin—I regard them as derived from the central larger nucleus of the cell.

Delage's observation that chambers arise from the fusion of several multinucleate groups, corroborates the account I have given of one of the methods of chamber formation (35, and *ante*, p. 312), though, as before said, we differ greatly in our views of the ultimate origin of such groups. But on the other hand I have repeatedly observed that chambers may also be formed by formative (amoeboid) cells which group themselves in hollow spheres. Some of these cells may contain but a single nucleus, while others contain more. Observations such as this would seem to disprove Delage's thesis that the colared cells are the immigrated ciliated cells of the larva.

Canal Epithelium.—Delage, like myself, finds that the canals arise independently of one another, as irregular spaces in the inner mass, that they gradually become lined with a definite epithelium and unite with one another and with the chambers to form a connected system. Regarding the origin of the canal epithelium, however, Delage entertains widely different views from my own. His account is as follows:

"The (once) ciliated cells surrounding the irregular spaces arrange themselves so as to form a nearly continuous wall, in which, here and there, an intermediary cell is found. This is, however, not the permanent epithelium, for intermediary cells lying outside it gradually take the place of the ciliated cells, which in their turn come to lie outside the definitive epithelial wall. Such ciliated cells, which have temporarily been occupied in lining the canals, now follow the example of their brethren and unite to form flagellated chambers."

Observations such as are embodied in my Pl. XVIII, Fig. 47, and Pl. XVII, Figs. 39 and 42, seem to me to contradict the above account. The canals shown in these figures are evidently just forming, and yet their walls are made up of elements which, to judge from Delage's figures, I must conclude he would regard as amoeboid and intermediary, certainly not as immigrated ciliated cells.

Finally, the distinction which Delage makes between intermediary and amoeboid cells, is to my own mind an artificial one. His amoeboid cells evidently correspond to my formative cells, but I find no special place for his "intermediary" group, because the plump formative cells are being constantly changed into elements which Delage would class as intermediary. An instance of this is found in the development of the dermal membrane, where formative cells are gradually transformed into the slender elongated cells forming the mesoderm of this membrane (*ante*, p. 307).

NOTE.—While this paper is passing through the press, a new contribution to the subject by Otto Maas appears.¹ The author has studied a large number of marine cornacuspongiae and has worked over the development of Spongilla. His account of the metamorphosis for all these forms differs but little from his previous account of the metamorphosis of the *Esperia* larva. In some points Maas differs from Delage's recent conclusions. Thus Maas does not find that the ciliated cells of the larva are engulfed by the amoeboids and subsequently liberated. No such peculiar association of the two kinds of cells occurs. With this I thoroughly agree, although differing entirely with Maas in the general view of the metamorphosis. Again Maas states that in those larvae with a "bare" posterior pole, like

¹ Die Embryonal-Entwicklung und Metamorphose der Cornacuspongien. *Zoolog. Jahrbücher*, Abth. für Anat. und Ontogenie. Bd. VII., 2. H.

that of *Esperella*, the immigration of the ciliated cells does not take place in spots anywhere over the surface, but the inner layer actually *overgrows* the layer of ciliated cells from the posterior pole forward. In a sectional figure of a metamorphosing larva (Pl. XX, Fig. 19) he represents the layer of ciliated cells as *overlapped* by the inner mass for a considerable distance. I have cut many sections through similar stages, but have never seen a trace of such overlapping. But I can understand how a section through a larva, whose surface had been pitted in on opposite sides, could give rise to such a figure. Such pitting in of the surface may occur when the fixing fluid permits the larva to contract in the moment of death. And I am inclined to believe that the section figured was made through such a larva.

It is, however, always venturesome to suggest a new interpretation of another's figures. The suggestion is not often happy. This is certainly true of Maas's intimation that my figures of "segmenting" gemmules (Notes on the Development of some Sponges, JOURNAL OF MORPHOLOGY, 1891,) indicate that the bodies in question are not gemmules but eggs, and the "segmentation" is a real segmentation. A glance at the series of figures given in this paper, illustrating the development of the gemmule of *Esperella*, will show that such an interpretation is impossible.

The author's renewed study of *Spongilla* has led him to abandon his former views (for a statement of which see p. 360) on the development of this sponge. He finds that the flagellated chambers and exhalent canals do not arise as diverticula from a central cavity, and that the inhalent canals are not formed as ectodermic invaginations, but that the whole development agrees substantially with that of the marine *cornacuspongiae*, as described by himself and Delage. My statement, therefore (p. 360), that Maas "brings *Spongilla* in line with those forms having a rhagon" is interesting only historically.

On pp. 301 and 368 I cite Maas's observations on the flattening of the ectoderm of the *Spongilla* larva and its transformation into that of the adult, as a strong argument for the universality of this phenomenon in the sponges. But the author's recent paper makes these citations antiquated, since he now believes that no such transformation takes place.

CHAPEL HILL, N.C., June 15, 1894.

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EXPLANATION OF THE PLATES.

Most of the drawings were made with the camera. The lenses referred to in the description of the figures, are those of Zeiss, except when the focal distance of the objective is given in inches. The drawings have been reduced to four-sevenths original size.

Common Reference Letters used in the Figures.

<i>af. c.</i>	Afferent canal.	<i>mes. b.</i>	Mesoderm band.
<i>an. f. c.</i>	Anlage of flagellated chamber.	<i>mes. gr.</i>	Group of mesoderm cells.
<i>can.</i>	Canal.	<i>mn. m.</i>	Multinucleate mass.
<i>c. m.</i>	Solid anlage of flagellated chamber.	<i>m. p.</i>	Peripheral mesodermic process.
<i>cna. w.</i>	} Canal wall.	<i>mes.</i>	Mesoderm (in places, mesentoderm).
<i>(c. w.)</i>		<i>os.</i>	Osculum.
<i>c. ef. c.</i>	Central efferent canal.	<i>os. c.</i>	Oscular cavity.
<i>cu.</i>	Cuticle.	<i>o. ov.</i>	Ovarian egg.
<i>d. mem.</i>	Dermal membrane.	<i>ov. f.</i>	Follicle of ovum.
<i>deg. f. c.</i>	Degenerated flagellated chamber.	<i>p. w.</i>	Partition wall.
<i>ef. c.</i>	Efferent canal.	<i>pr. g.</i>	Problematical gemmule-like bodies.
<i>ect. (ec.)</i>	Ectoderm.	<i>p. p.</i>	Posterior pole of larva.
<i>ec. m.</i>	} Ectodermal membrane.	<i>pr. th.</i>	Problematical thickenings.
<i>(ec. mem.)</i>		<i>p. for.</i>	Perforation through peripheral mesodermic zone.
<i>ec. un. p. p.</i>	Ectoderm of unpigmented pole.	<i>p. z.</i>	Peripheral mesodermic zone.
<i>f. c.</i>	Flagellated chamber.	<i>p. c.</i>	Pale cell.
<i>for. c. g.</i>	Solid group of formative cells.	<i>r.</i>	Ridge.
<i>f.</i>	Furrow.	<i>r. g.</i>	Mature gemmule.
<i>g.</i>	Gemmule.	<i>s. d. c.</i>	Subdermal cavity.
<i>g. f.</i>	Follicle of gemmule.	<i>sh.</i>	Gemmule sheath.
<i>g. sh.</i>	Sheath of gemmule.	<i>sup. ef. c.</i>	Superficial efferent canal.
<i>gr. c.</i>	Granular cell.	<i>sp.</i>	Spicule.
<i>In. sp.</i>	Intercellular space.	<i>sp. p.</i>	Spicular pole.
<i>l.</i>	Larva.	<i>s. p.</i>	Surface pore.

EXPLANATION OF PLATE XIV.

*(Esperella fibrexilis.)*FIG. 1. *Esperella fibrexilis*. $\times 1\frac{1}{2}$.FIG. 2. Vertical section of *Esperella*, showing canal system and skeleton. A.4.FIG. 3. Spicules. *a*, oxytyle; *b*, modification of the same; *c*, after treatment with caustic potash; *d*, toxaspire; *e*, sigma; *f*, sigmaspire. D.4.FIG. 3'. *a*, *b*, *c*, views of small shovels: *a*, somewhat from the end; *b*, face view; *c*, side view. *d*, a small *sigma* which will develop into a shovel. $\times 900$.FIG. 3''. Large shovels—*a*, face view; *b*, side view; *d.l.* dorsal lobe; *v.l.*, ventral lobe; *l.n.* lateral notch; *t.*, tooth. $\times 900$.

FIG. 4. Dermal membrane. A.4.

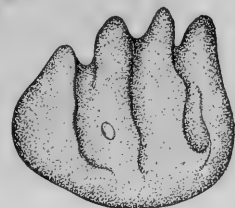
FIG. 5. Surface of adult. 4-inch objective.

FIG. 6. Ditto, showing osculum and oscular cavity.

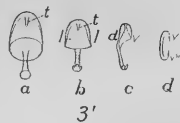
FIG. 7. Surface of adult. 4-inch objective.

FIG. 8. Section: gemmules in mesoderm of parent. $\times 800$.

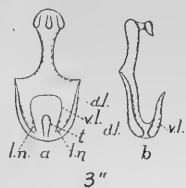
FIG. 8'. Different conditions of the nucleus in the gemmule cells.



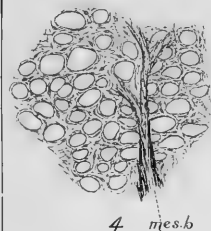
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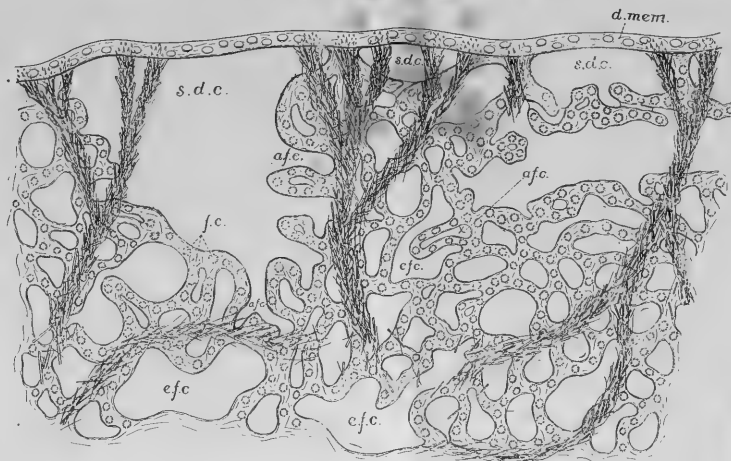
3'



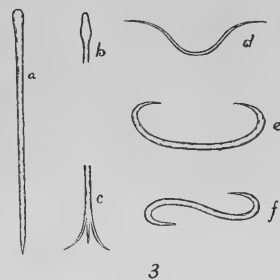
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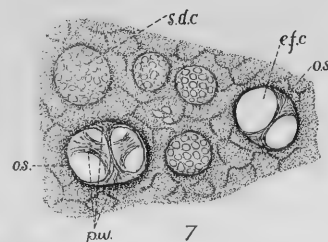
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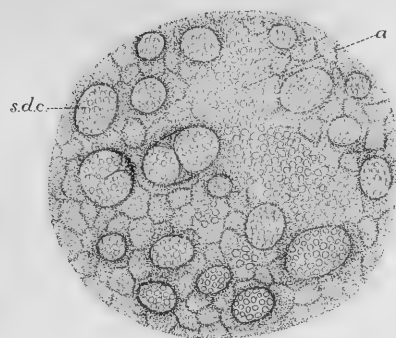
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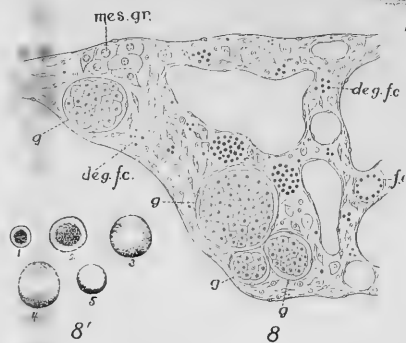
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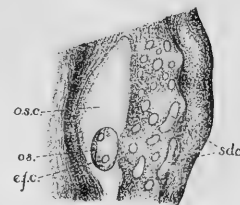
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5



8



6

EXPLANATION OF PLATE XV.

(Esperella fibrexilis.)

FIG. 9. Section: gemmules in mesoderm of parent. $\times 800$.

FIG. 10. Section, showing possible origin of a gemmule from a single cell. $\times 800$.

FIG. 11. Section: gemmules in mesoderm of parent.

FIG. 12. Section, showing situation of young and ripe gemmules in body of parent. A.4.

FIG. 13. Section: adult tissue with gemmules and cell groups. The group *a* probably derived from simple cell. $\times 800$.

FIG. 14. Section: adult tissue with cell group and problematical gemmule-like body. $\times 800$.

FIG. 15. Section: adult tissue with cell groups (gemmule anlagen). D.4.

FIG. 16. Section, indicating the fusion of gemmules. $\times 800$.

FIG. 17. Section: adult tissue with gemmules. Gemmule *x* has probably been formed by fusion. D.4.

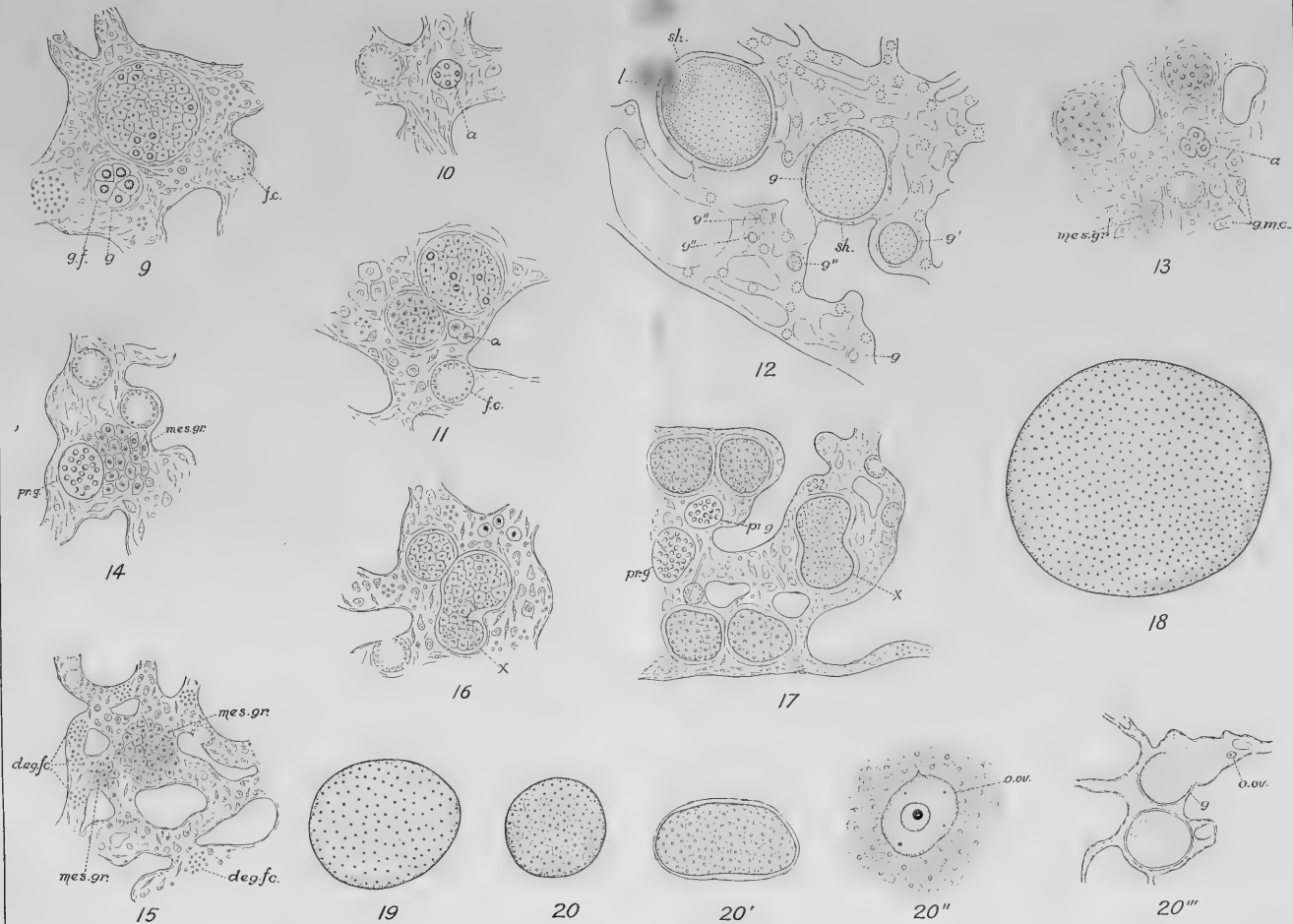
FIG. 18. Section through mature gemmule. D.4.

FIG. 19. Section through gemmule of about half the full size. D.4.

FIG. 20, 20'. Sections through immature gemmules. D.4.

FIG. 20''. Ovarian egg surrounded by mesoderm of parent. F.4.

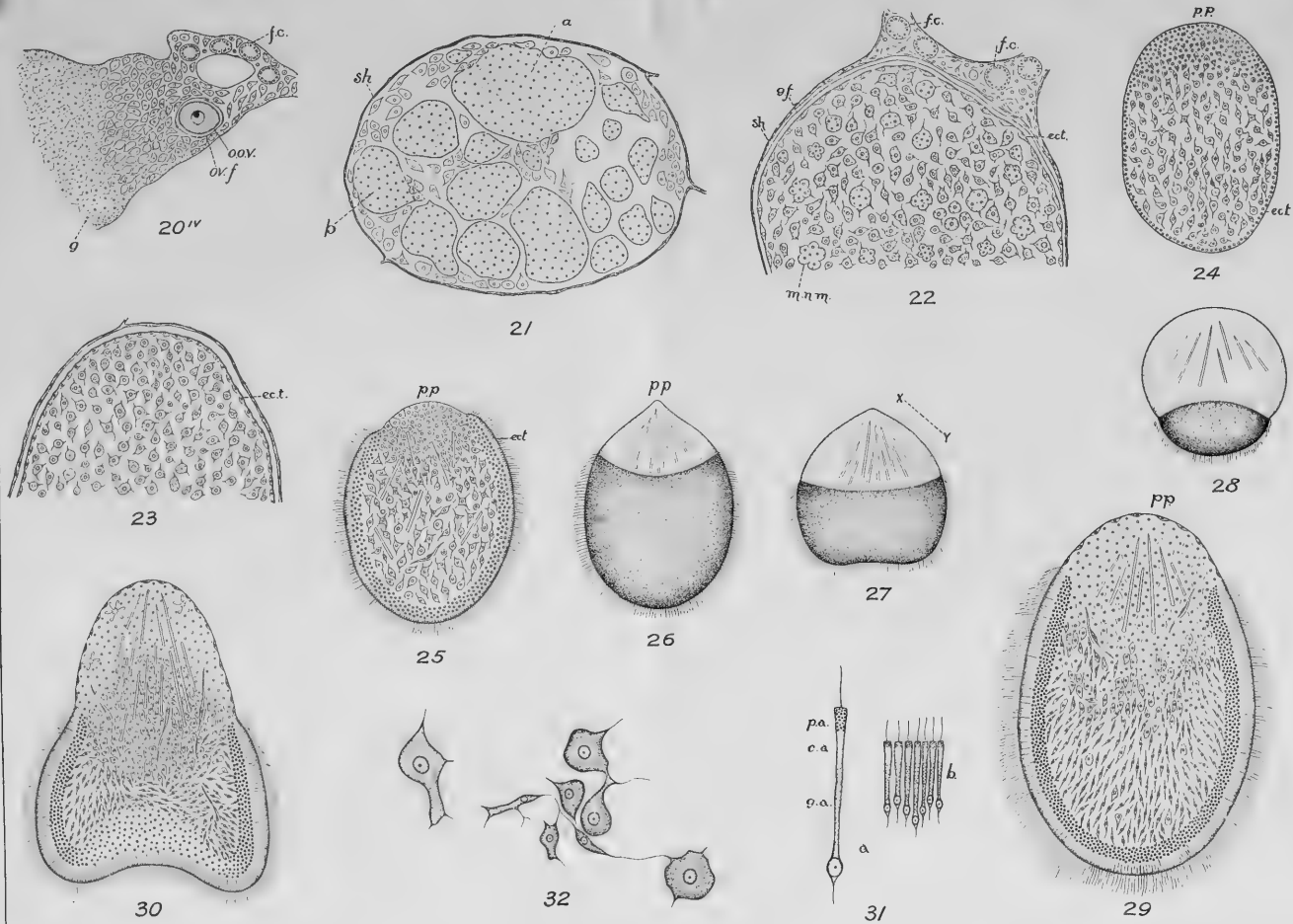
FIG. 20'''. Adult tissue with two full-sized gemmules, one of which, *g*, is continuous with the external mesoderm, in which is an ovarian egg, *o.ov*. A.4.



EXPLANATION OF PLATE XVI.

(Esperella fibrexilis.)

- FIG. 20^{iv}. The ovarian egg of Fig. 20^{'''}, with neighboring tissue. D.4.
FIG. 21. Gemmule breaking up or "segmenting." Section. D.4.
FIG. 22. Section through a gemmule near the close of "segmentation." D.4.
FIG. 23. Part of a section through a gemmule, entirely broken up into separate cells. D.4.
FIG. 24. Longitudinal section through gemmule, in which the posterior pole is differentiating. C.4.
FIG. 25. Longitudinal section through gemmule larva, still in body of parent. C.4.
FIG. 26. Surface view of swimming larva shortly after birth. A.4.
FIG. 27. Surface view of swimming larva 36 hours after birth. A.4.
FIG. 28. Surface view of incompletely metamorphosed larva. A.4.
FIG. 29. Longitudinal section of larva shortly after birth. D.4.
FIG. 30. Longitudinal section of older larva. D.4.
FIG. 31. Ectoderm cells of swimming larva — maceration products.
FIG. 32. Parenchyma cells of swimming larva — maceration products.



EXPLANATION OF PLATE XVII.

(Esperella fibrexilis.)

FIG. 33. Ectoderm of posterior pole and adjoining parenchyma of swimming larva—maceration product.

FIG. 34. Rosette group of sigma spicules from swimming larva—seen in optical section.

FIG. 36. Longitudinal section through incompletely metamorphosed larva. D.4.

FIG. 37. Vertical section of sponge attached to surface film of water. D.4.

FIG. 38. Vertical section of recently attached sponge. A.4, tube out.

FIG. 39. Peripheral part of section through recently attached sponge. D.4.

FIGS. 40, 41. Parts of sections similar to the preceding, showing anlagen of flagellated chambers. D.4, tube out.

FIG. 42. Part of section through recently attached sponge, showing formation of canals and flagellated chambers. D.4.

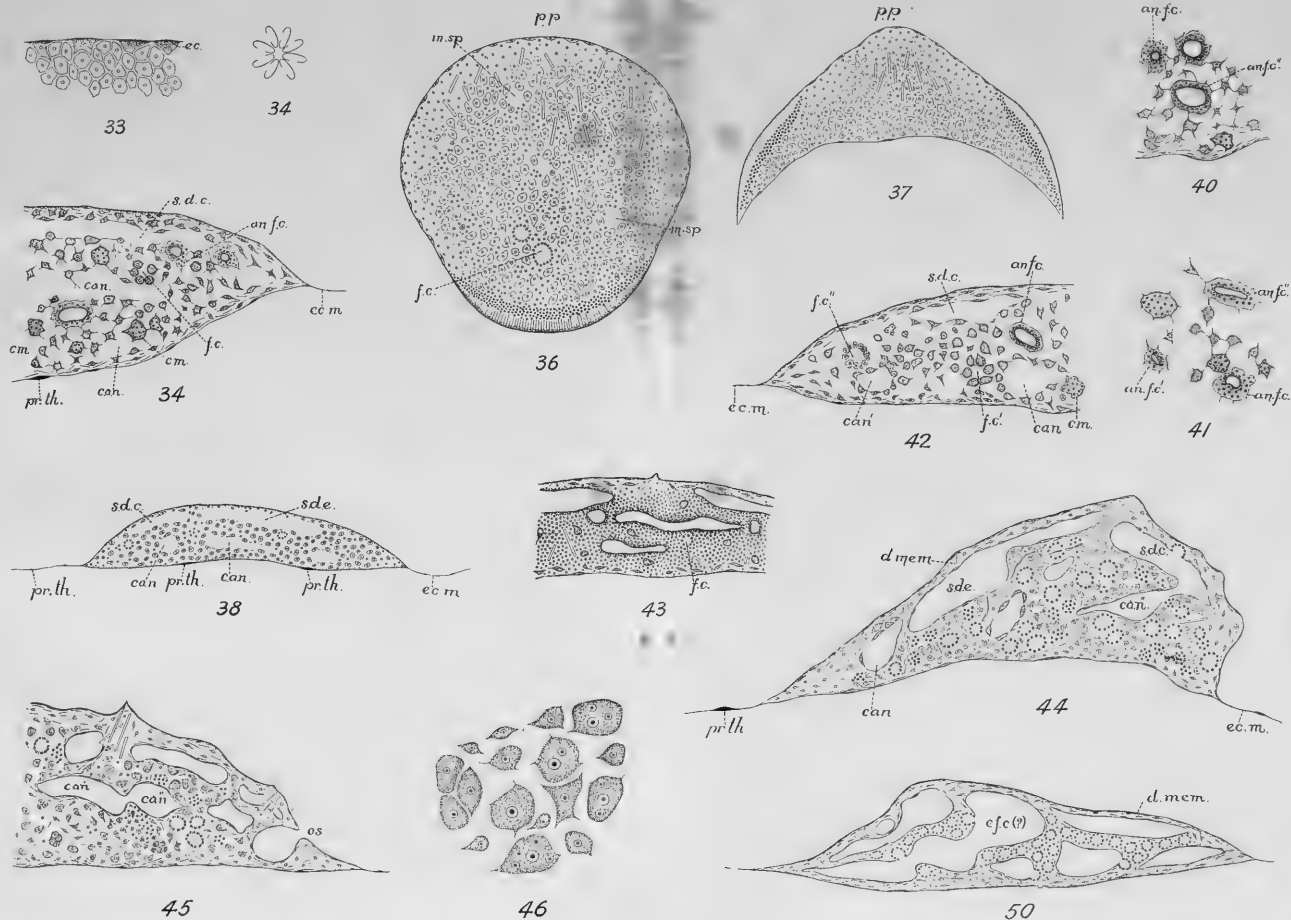
FIG. 43. Part of section through young sponge, the mesoderm of which consists almost entirely of fine cells. D.4.

FIG. 44. Vertical section through young sponge—subdermal cavities, canals and chambers completely differentiated. D.4.

FIG. 45. Vertical section through a young sponge. D.4.

FIG. 46. Group of multinucleate mes-entoderm cells.

FIG. 50. Section of a young sponge—for canal system. C.4.



EXPLANATION OF PLATE XVIII.

(Esperella fibrexilis.)

FIG. 47. Part of section through young sponge, showing formation of canal wall. $\frac{1}{12}$ Immersion 4.

FIG. 48. Section of young sponge, showing osculum. D.4.

FIG. 49. Section through peripheral part of young sponge. D.4.

FIGS. 51, 52, 53. Sections of young sponges, for canal system. C.4.

FIG. 54. Surface view of young sponge with surrounding ectodermal membrane covered with debris. A.4.

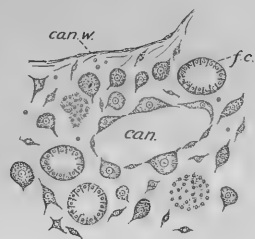
FIG. 55. Combined surface view and horizontal optical section of young sponge — note abundance of formative cells and absence of chambers. A.4.

FIG. 56. Horizontal optical section of peripheral part of young sponge — formation of peripheral mesodermic zone and canals. D.4.

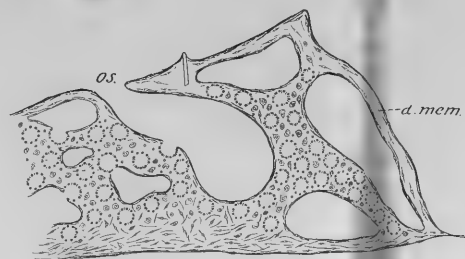
FIG. 57. Horizontal optical section of older sponge in which flagellated chambers have formed. D.4.

FIG. 58. Surface view of sponge with openings into subdermal spaces. A.4.

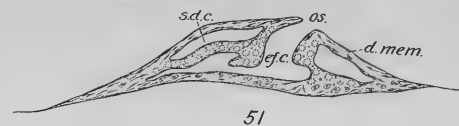
FIG. 59. Part of periphery of preceding figure, to show pores. D.4.



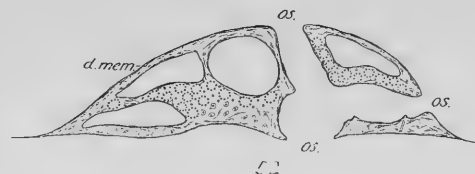
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48



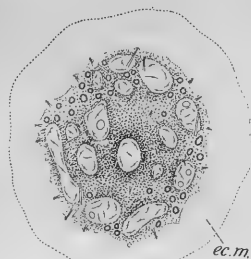
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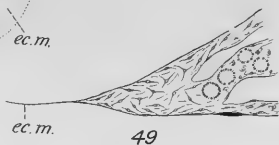
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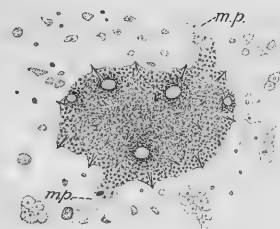
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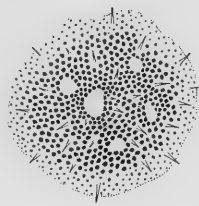
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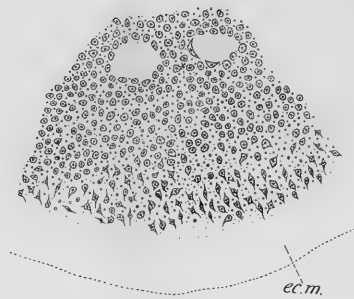
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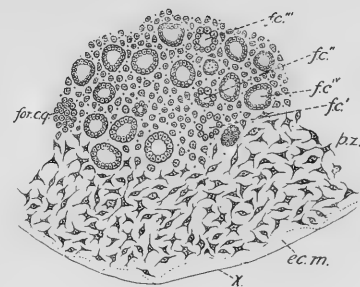
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56



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58





EXPLANATION OF PLATE XIX.

(Tedania Brucei.)

FIG. 60. Portion of surface of adult, showing the meandering ridges and furrows. $\times 8$.

FIG. 61. Section of adult, vertical to surface—arrangement of gelatinous and spongy tissue, and skeleton. $\times 10$.

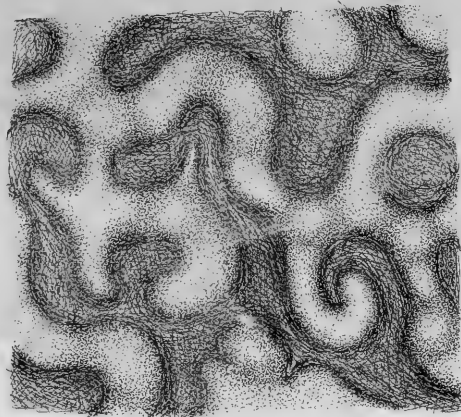
FIG. 62. Transverse section through base of oscular papilla. $\times 5$.

FIG. 63. Transverse section near oscular end of young sponge, 6 inches high. $\times 10$.

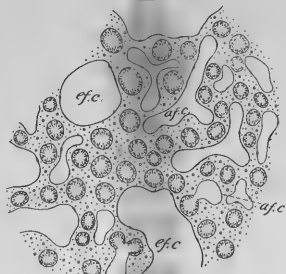
FIG. 64. Vertical section of adult—arrangement of canals in gelatinous and spongy regions. A.4.

FIG. 65. Section of adult—disposition of the ultimate branches of the canals. D.4.

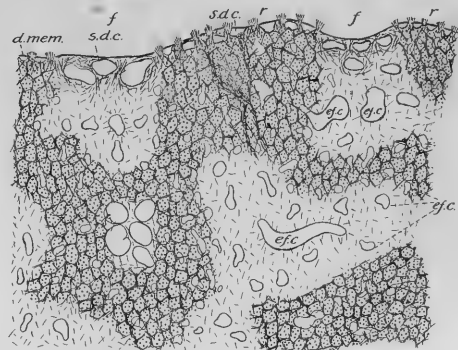
FIG. 66. Dermal membrane. D.4.



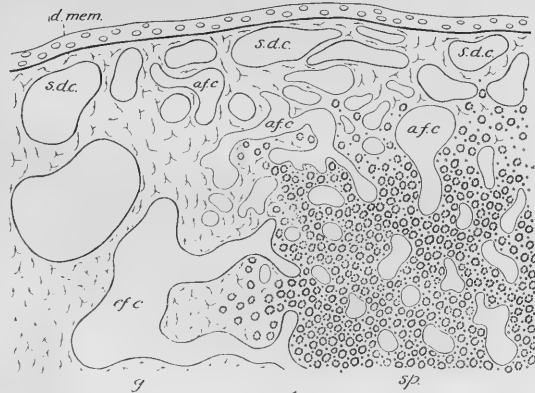
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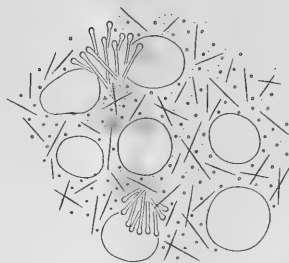
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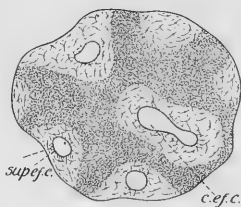
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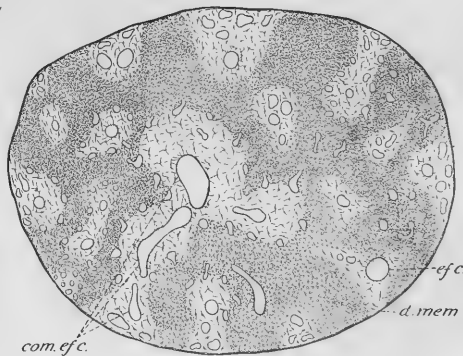
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66



63



62

EXPLANATION OF PLATE XX.

(Tedania Brucei.)

FIG. 67. Spicules: *a*, slightly bent stronglyloxea; *b*, oxea; *c*, tylote. (*a*, $\frac{2.0}{100}$ mm.)

FIG. 68. Surface view, showing orifices intermediate in size between pores and oscula. $\times 8$.

FIG. 69. Section showing bodies *g*, probably young gemmules, imbedded in mesoderm. $\times 900$.

FIG. 70. Section: adult tissue with part of mature gemmule and a young gemmule. D.4.

FIG. 71. Section: mature gemmule with surrounding tissue. A.4.

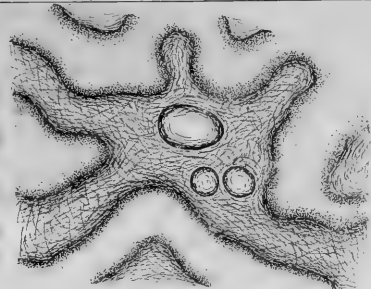
FIG. 72. Section through gemmule beginning to "segment." C.4.

FIG. 73. Part of section through gemmule, completely broken up into masses. D.4.

FIG. 74. Part of section through gemmule near the close of "segmentation." C.4.

FIG. 76. Section through embryo — columnar ectoderm entirely covers embryo. A.4.

FIG. 77. Longitudinal section through unpigmented pole of embryo — cells at this pole still columnar. D.4.



68



a

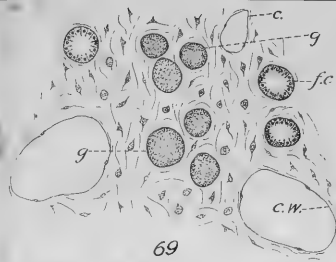


b

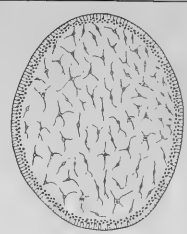


c

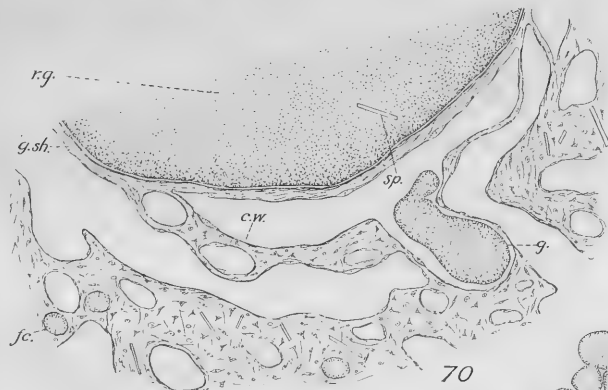
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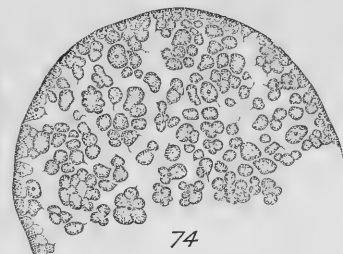
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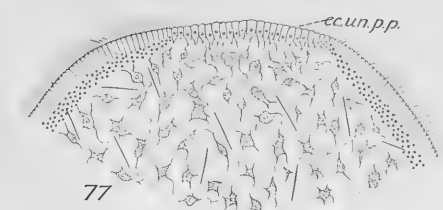
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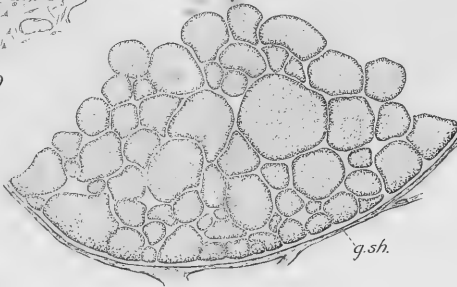
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73



72



EXPLANATION OF PLATE XXI.

(Tedania Brucei.)

FIG. 75. Section of gemmule, in which the ectoderm layer is clearly differentiated. D.4.

FIG. 76'. Part of Fig. 76. D.4.

FIG. 78. Longitudinal section through unpigmented pole of swimming larva, shortly after birth. D.4.

FIG. 79. Surface view of larva just born. A.4.

FIG. 80. Surface view of older larva — unpigmented "plug" now conspicuous. A.4.

FIG. 81. Longitudinal section through swimming larva, a day old. C.4.

FIG. 82. Attached larva with spicular pole pulled in. A.4.

FIG. 83. Vertical section through sponge just attached. Ciliated ectoderm entirely metamorphosed, unpigmented pole still recognizable. C.4.

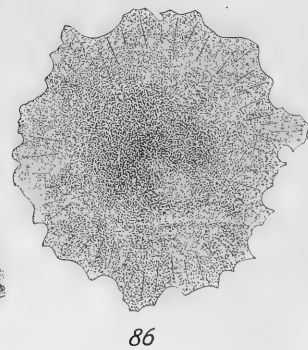
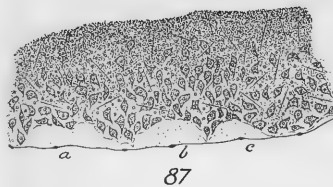
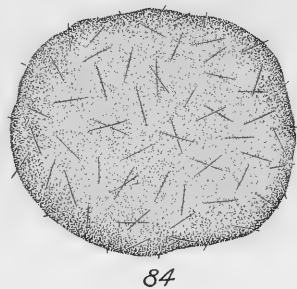
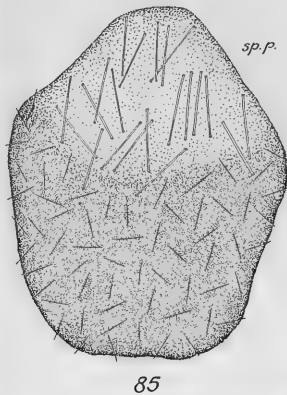
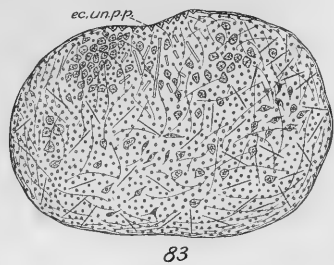
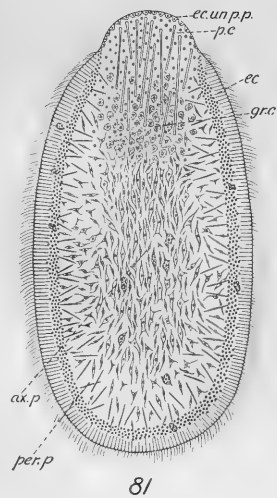
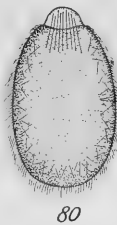
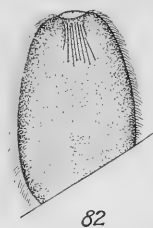
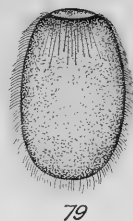
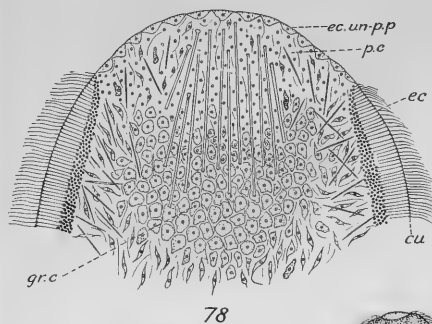
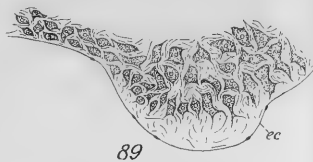
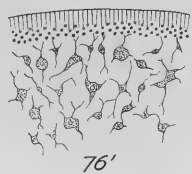
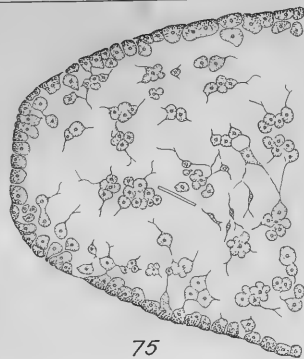
FIG. 84. Surface view of recently attached sponge — spicular pole lost. A.4.

FIG. 85. Surface view of sponge just attached — shows how spicular pole loses its identity — spicules are being distributed to various parts of sponge. A.4.

FIG. 86. Surface view. Sponge is solid, and margin is thrown into irregular lobes (first stage in formation of ectodermal membrane). A.4.

FIG. 87. Part of periphery of preceding figure. D.4.

FIG. 89. Part of periphery of Fig. 88. D.4.



EXPLANATION OF PLATE XXII.

(Tedania Brucci.)

FIG. 88. Surface view. Cavities have formed in sponge, and mesoderm no longer extends to edge of body. A.4.

FIG. 90. Surface view — ectodermal membrane well developed except in one region — central canal. A.4.

FIG. 91. Surface view — sponge of very irregular shape, which is, however, common. 1-inch objective.

FIG. 92. Vertical section of recently attached sponge — no ectodermal membrane. C.4.

FIG. 93. Vertical section of sponge with developed canal system. C.4.

FIG. 94. Peripheral region of same sponge — double nature of ectodermal membrane. D.4.

(Tedanione foetida.)

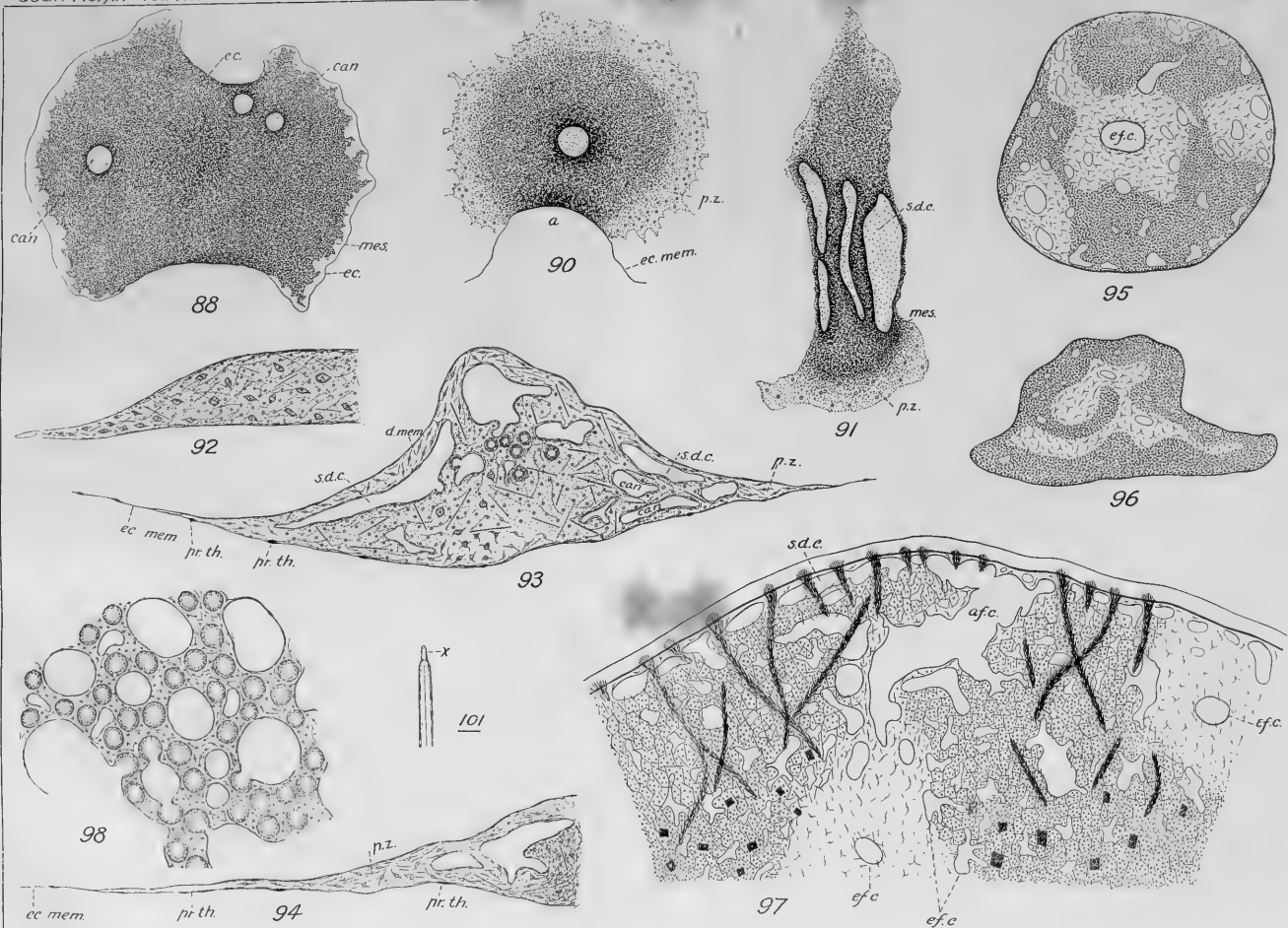
FIG. 95. Section through oscular papilla. $\times 4$.

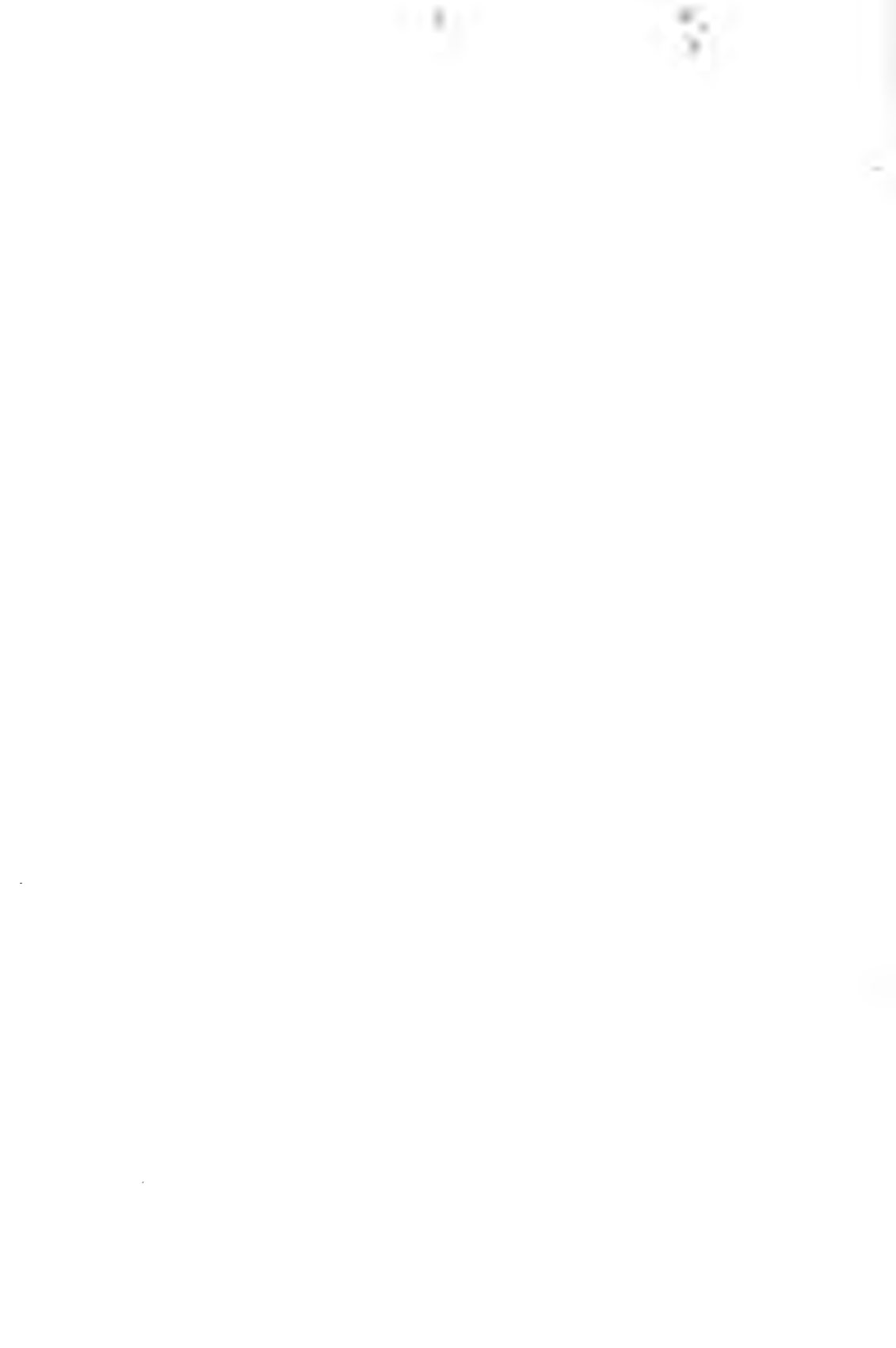
FIG. 96. Vertical section through body of sponge. $\times 3$.

FIG. 97. Vertical section of adult — canal system and arrangement of skeleton. 3-inch objective.

FIG. 98. Section of adult — disposition of ultimate branches of canals. D.4.

FIG. 101. Modified oxea — very common form of spicule.





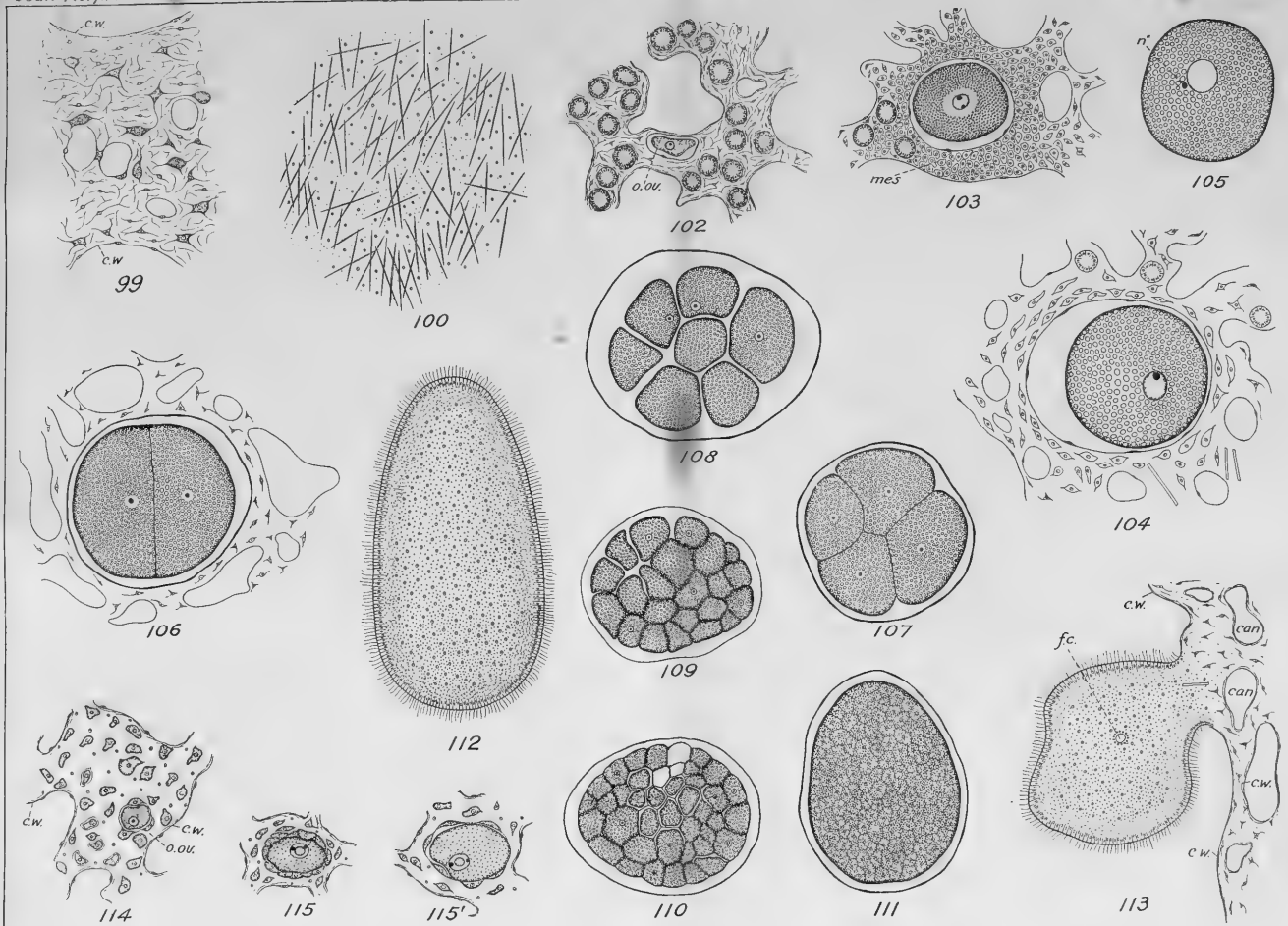
EXPLANATION OF PLATE XXIII.

(Tedanione foetida.)

- FIG. 99. Section of adult — histology of gelatinous tissue. D.4.
FIG. 100. Dermal membrane. A.4.
FIG. 102. Section showing very young egg-cell. D.4.
FIG. 103. Section showing immature egg surrounded by numerous mesoderm cells. D.4.
FIG. 104. Section of adult tissue with egg of full size — only one nucleolus. D.4.
FIG. 105. Section of mature egg — extruded nucleolus. D.4.
FIGS. 106–110. Sections of segmenting eggs. D.4.
FIG. 111. Section of planula — coarse yolk has disappeared. D.4.
FIG. 112. Longitudinal section of swimming larva. D.4.
FIG. 113. Section showing a larva attached to canal-wall of mother. D.4.

(Hircinia acuta.)

- FIG. 114. Section of adult tissue with young ovum. D.4.
FIGS. 115, 115'. Sections showing young ova and follicles. D.4.



EXPLANATION OF PLATE XXIV.

(Hircinia acuta.)

FIG. 116. Section of immature ovum, surrounded by numerous mesoderm cells. D.4.

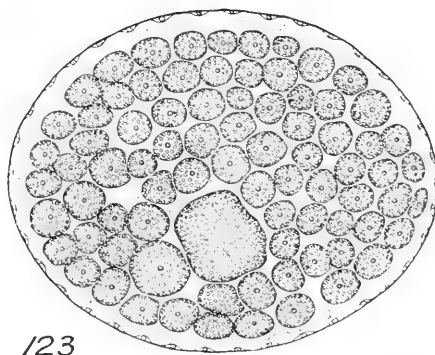
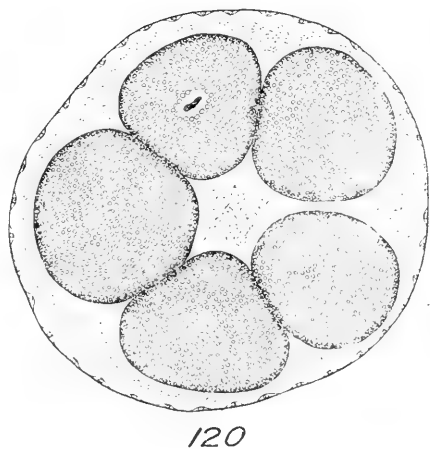
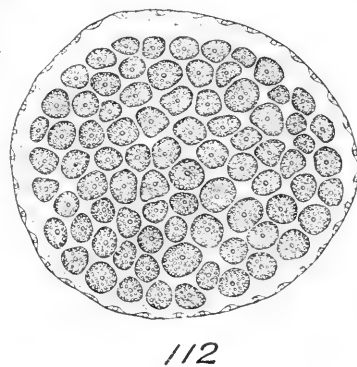
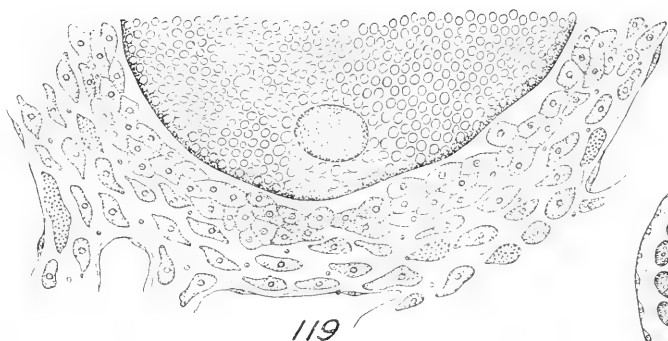
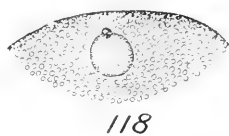
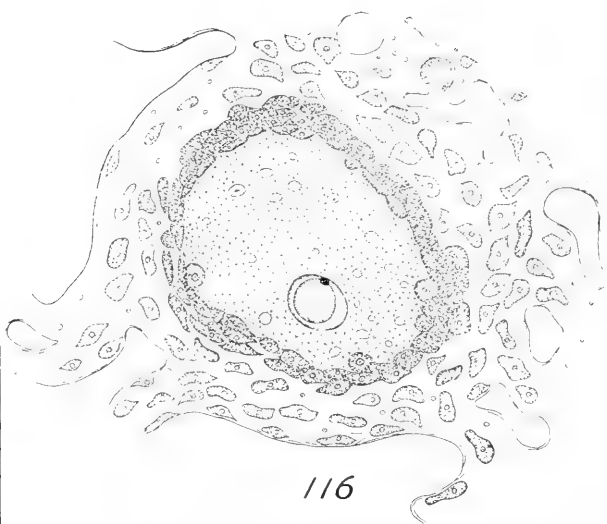
FIG. 117. Section showing mature (as to size) ovum in situ. A.4.

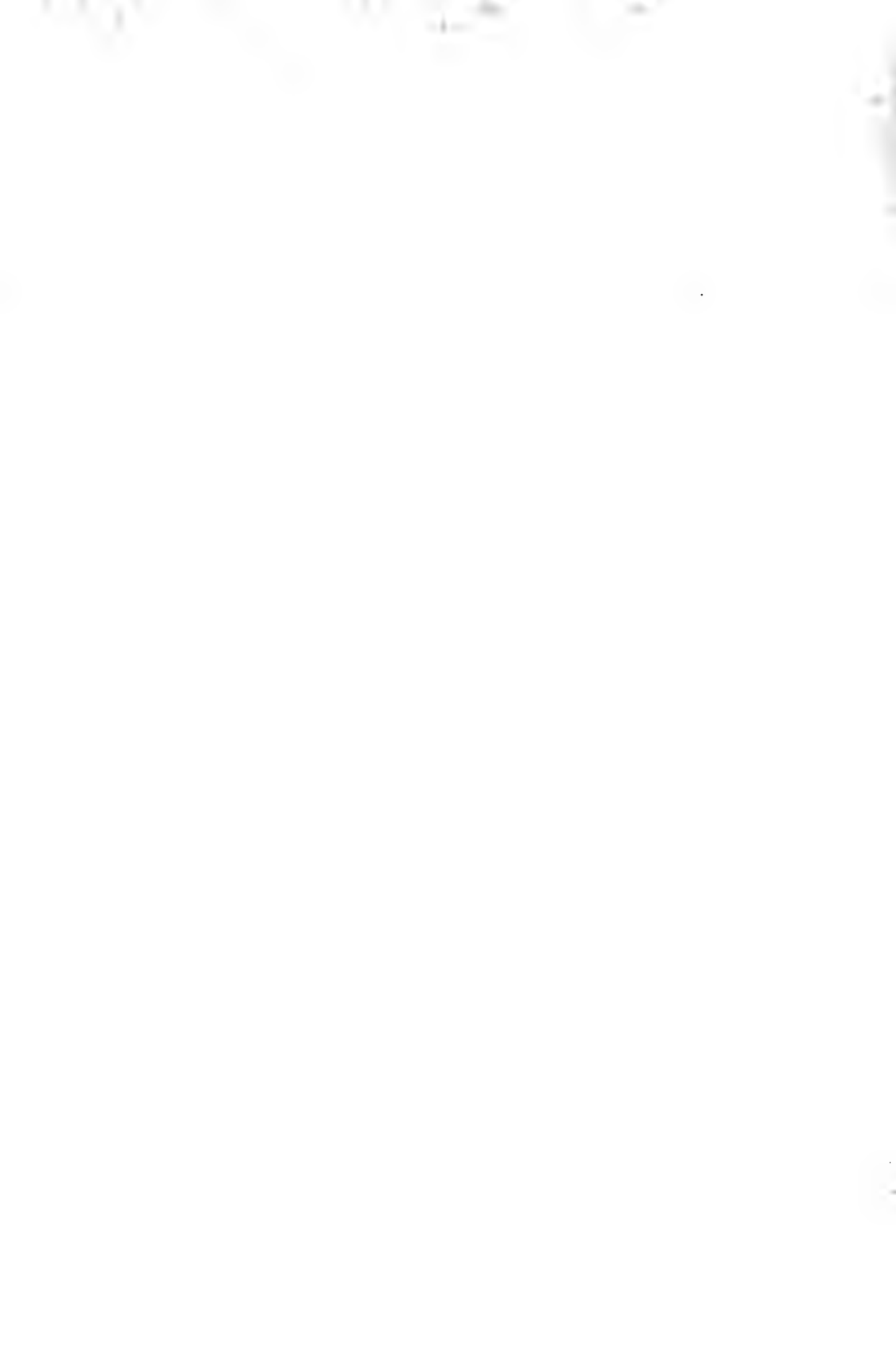
FIGS. 118, 118'. Two sections of mature ova showing stages in the extrusion of the second nucleolus. D.4.

FIG. 119. Section through part of ripe egg with surrounding follicle. D.4.

FIG. 120. Section through segmenting egg. C.4.

FIGS. 121, 122. Sections of morulas. C.4.





EXPLANATION OF PLATE XXV.

(Fig. 5 original — the other figures borrowed to illustrate the section "Remarks on the Morphology of Sponges.")

FIG. 1. *Anamixilla torresi*. Trans. section, from Polejaeff, Challenger Report on Calcareous, Pl. IV, Fig. 2a.

FIG. 2. *Leucilla connexiva*. Trans. section, from Polejaeff, Pl. VI, Fig. 1a.

FIG. 3. *Leucilla uter*. Trans. section, from Polejaeff, Pl. VI, Fig. 2a.

FIG. 4. *Leuconia multiformis*. Trans. section, from Polejaeff, Pl. VI, Fig. 3a.

FIG. 5. Diagrammatic section of an hypothetical silicious sponge.

FIG. 6. *Leucandra caminus*. From Vosmaer's Spongien, Taf. I, Fig. 18, after Haeckel.

FIG. 7. *Plakina monolopha*. Vertical section, from Schulze. Zeit. für Wiss. Zool., 34. Bd., Taf. XX, Fig. 4.

FIG. 8. Young *Plakina monolopha*, just attached. Vertical section, from Schulze, l. c.

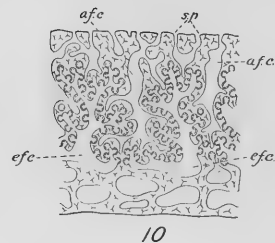
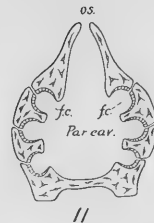
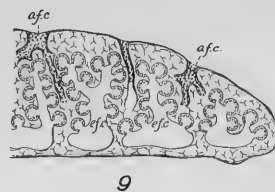
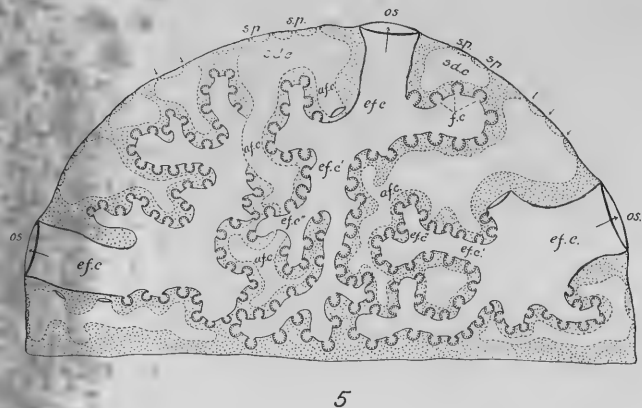
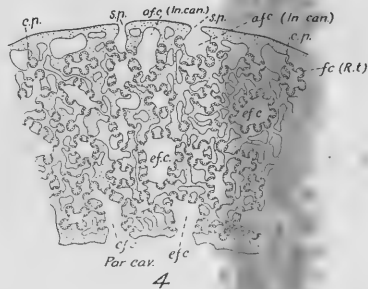
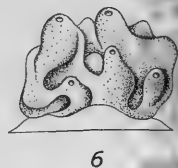
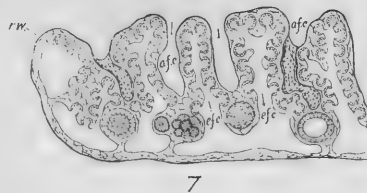
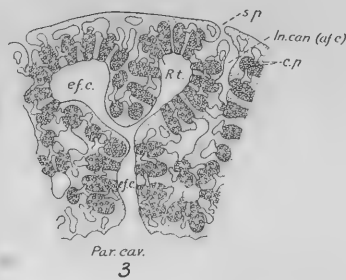
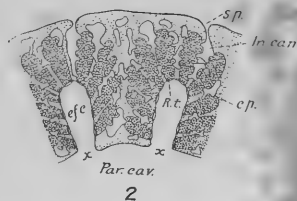
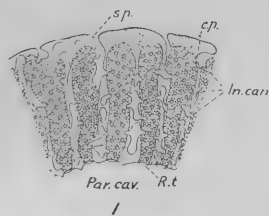
FIG. 9. *Plakina dilopha*. Vertical section, from Schulze, l. c. Taf. XX, Fig. 11.

FIG. 10. *Plakina trilopha*. Vertical section, from Schulze, l. c. Taf. XXI, Fig. 12.

FIG. 11. Rhagon stage of *Oscarella lobularis*, after Heider, from Korschelt und Heider's Lehrbuch, p. 6.

REFERENCE LETTERS TO THIS PLATE.

<i>af. c.</i>	Afferent canal.	<i>mes.</i>	Mesoderm.
<i>c. p.</i>	Chamber pore.	<i>os.</i>	Osculum.
<i>ec.</i>	Ectoderm.	<i>Par. cav.</i>	Paragastric cavity.
<i>en.</i>	Entoderm.	<i>r. w.</i>	"Ringwall."
<i>ef. c.</i>	Efferent canal.	<i>R. t.</i>	Radial tube.
<i>f. c.</i>	Flagellated chamber.	<i>s. p.</i>	Surface pore.
<i>In. can.</i>	Intercanal.		



ON THE CLEAVAGE OF AMPHIBIAN OVA.

E. O. JORDAN AND A. C. EYCLESHYMER.

OUR observations upon the early cleavage stages of several sorts of amphibian eggs were made primarily with a view to determining the extent and frequency of cleavage variation. The eggs examined were those of two species of Urodela, — *Amblystoma punctatum* and *Diemyctylus viridescens* — and two Anura, — *Rana palustris* and *Bufo variabilis*.

The unsegmented eggs of *Amblystoma* were usually collected before eight o'clock in the morning, and began to segment at three or four o'clock on the afternoon of the same day. As we hope to show presently, there is reason to believe that there is an interval of nearly ten hours between the laying of the eggs and the appearance of the first furrow; these eggs, therefore, were probably laid at about five or six o'clock in the morning. Other bunches of *Amblystoma* eggs were in the early stages of cleavage when collected in the morning, and these were undoubtedly deposited on the preceding evening. Clarke ('80) states that most of the eggs laid by the *Amblystoma* in his aquaria "were laid during the night, and by nine o'clock the next morning the first segmentation furrow had usually made its appearance."

The eggs of *Diemyctylus* are best obtained from females in captivity, and can be removed from the membrane as soon as laid (Jordan, '93, p. 275 *et seq.*). The first cleavage furrow appears about ten hours after laying.¹ Since there is a close correspondence in all other time phenomena between the egg of the newt and the egg of *Amblystoma*, we are justified in

¹ Born (*Anat. Anz.* VII, 1892, p. 809) refers to a paper by Grönroos, which we have, unfortunately, been unable to see (Ueber die Eifurchung bei den Tritonen. Diss. inaug. Helsingfors, 1890). According to Born, Grönroos has shown "dass bei gewöhnlicher Zimmertemperatur die erste Furche an reifen Tritoneiern 5-6 Stunden nach der Besamung auftritt — gemeint sind natürlich Uteruseier — im Gegensatz zu den Eiern unserer einheimischen Anuran bei denen unter gleichen Bedingungen der Eintritt der ersten Furche nach 3 Stunden erfolgt."

concluding, as we have already intimated, that in this respect also, there is time agreement, and that the eggs of *Amblystoma*, as well as those of the newt, begin to segment about ten hours after they are laid.

The eggs of *Rana* and *Bufo* may be found unsegmented in the ponds in the early morning. The first furrow appears, as a rule, earlier in the day than it does in *Amblystoma*. We did not particularly question this point, however, since Newport (Phil. Trans. 1851, 1853, 1854) long ago determined that the frog's egg begins to segment in from four to five hours after the spermatozoa are applied. Our own incidental observations upon the eggs of the frog and toad amply confirm this result.

All the eggs when brought to the laboratory were put under uniform conditions — in shallow dishes of water at about 18° C. The cleavage stages were followed in the living egg with the aid of the Zeiss dissecting microscope. A plane mirror placed underneath the flat and thin-walled glass dish in which the egg rested enabled us to observe accurately the progress and position of the furrows in the lower hemisphere.

The exact time of appearance of each set of furrows was noted in all cases, and is recorded on most of our figures. The following table expresses this record in a condensed form, the times given for the inter-cleavage periods representing the average of our observations on some fifteen to twenty eggs in each stage.¹

	<i>Amblystoma</i> .	<i>Rana</i> .	<i>Diemyctylus</i> .	<i>Bufo</i> .
Interval between fertilization and 1st cleavage.	10 hrs. (?)	4-5 hrs.	10 hrs.	4-5 hrs.
Interval between 1st and 2d cleavages . . .	1 hr. 50 m.	1 hr. 15 m.	2 hrs.	1 hr. 5 m.
Interval between 2d and 3d cleavages . . .	1 hr. 55 m.	1 hr. 15 m.	1 hr. 45 m.	1 hr.
Interval between 3d and 4th cleavages . . .	2 hrs.	1 hr.	1 hr. 40 m.	1 hr.
Interval between 4th and 5th cleavages . . .	1 hr. 40 m.	1 hr. 50 m.	1 hr.
Interval between 5th and 6th cleavages . . .	[1 hr. 35 m.]	[2 hrs. 45 m.]
Interval between 6th and 7th cleavages . . .	[1 hr. 25 m.]	[2 hrs. 45 m.]
Interval between 7th and 8th cleavages . . .	[1 hr. 25 m.]
Interval between 8th and 9th cleavages . . .	[1 hr. 25 m.]
Interval between 9th and 10th cleavages . . .	[1 hr. 30 m.]

¹ The times enclosed in brackets refer to individual cases, and are not averages. The *Amblystoma* egg, in the instance recorded, happened to have a quicker rhythm than the majority, and the *Diemyctylus* egg a slower rhythm.

Each egg, as a rule, possesses an individual rhythm of cell-division, and the time intervals between the different sets of furrows are substantially the same in the same egg. There is, however, considerable variation between these rhythms in different eggs. We have, for example, observed the fourth set of furrows in an *Amblystoma* egg follow the third set in one hour and thirty minutes, while in another egg from the same mother the fourth set appeared only after an interval of two hours and forty-five minutes.

Making all due allowance, however, for this asynchronism, one fact stands out prominently in the table above. This is the fact that the inter-cleavage periods in the Urodelan eggs are much longer than the corresponding periods in the Anuran eggs. This difference does not depend upon the size of the egg. The small egg of the newt and the large egg of *Amblystoma* have practically the same rapidity of cell-division. The egg of *Rana*, on the other hand, which is considerably larger than the egg of the newt, divides much more speedily than the latter. We see no escape, therefore, from the conclusion that in this instance the rapidity of cell-division depends upon the innate and inherited tendencies of the cytoplasm and nucleus, rather than upon the size of the ovum. This view receives confirmation from the fact that the whole course of Urodelan and Anuran development is marked by a similar disparity in time. The formation and closure of the blastopore, the formation and closure of the neural folds, all take place more expeditiously in the Anuran than in the Urodelan embryos, while the Anuran tadpole frees itself from its gelatinous envelopes at a time when the Urodelan tadpole of the same age is destined to remain imprisoned for some ten days. The whole course of Urodelan development, therefore, from the entrance of the sperm (see Jordan, '93) to the release of the larva, is slower than that of the Anuran. This difference in rapidity of cell-division extends, as we have shown, to the earliest cleavage stages, and is foreshadowed in the pre-cleavage phenomena.

The quantity of yolk, however, exerts an unmistakable influence upon the speed with which furrows cut their way to the

lower pole. In the largest eggs (*Amblystoma*) the vertical furrows consume nearly two hours in reaching the lower pole, while in the eggs of *Diemyctylus* but little over an hour is needed for the same operation. The furrows in the newt and in the frog, on the contrary, advance towards the lower pole with about equal velocity, although, as we have stated, the inter-cleavage period is much the longer in the newt.

We have already indicated in a preliminary note the more important varieties of cleavage that we observed (*Anat. Anz.* VII, 1892). The proportion of eggs that swerved from the Amphibian "type" somewhat surprised us. In a batch of seventy-one frog eggs from one mother, for example, the first furrow divided all the eggs into nearly equal hemispheres. The second furrow cut the first at nearly right angles in the median line in sixty-nine eggs. In the other two eggs furrows appeared as shown in Fig. 5; *these two eggs were isolated, and produced perfectly normal embryos*. The next furrow was truly horizontal in only twenty-nine out of the sixty-nine eggs remaining; the other forty showed considerable variation. In some of these forty eggs equatorial furrows appeared in three quadrants, and a true vertical in the other quadrant (Figs. 2 and 8). In other eggs three cleavage planes were vertical and one horizontal (Fig. 26). In still other eggs the whole third set of furrows was vertical (Fig. 33).

This large proportion of variations from the normal is not uncommon, although in other batches of eggs a smaller number of variations was usually observed. In this respect there is considerable difference between eggs from different parents. Eggs from some parents showed far greater tendency to vary in the early stages than did others. In general, about one half of the eggs fail to form a true "first equatorial" plane in all four quadrants. Some of the more interesting of these deviations from the type are shown in Plate XXVI.

As the number of cells increases the cell-divisions become less synchronous. Some cells show a tendency to divide more rapidly than others, and consequently the furrows do not all appear simultaneously, but follow one after the other at perceptible intervals. In the fifth and sixth set of furrows, for

example, there is a considerable lapse of time between the appearance of the first furrow in either set and the last furrow in the same set. Even in the third set we have observed so long a period as thirty minutes between the appearance of furrows in different quadrants (Figs. 12, 18). This tendency to divergent rhythm of cell-division brings it to pass that the last cell-division in one group falls nearer and nearer in time to the first cell-division in the next group, and in this way the distinction between "sets" of furrows is eventually lost. (See Figs. 4, 13, 19, 28, 32, etc.) Up to the point to which we have been able to follow the cleavage, however (*Amblystoma* 10th set), the general synchronism of division is maintained, and the furrows appear closely one after the other; then there is a marked pause before the next cell-division.

The question as to the "homology" of the third Teleostean furrow with the first equatorial furrow of the Amphibian egg has given rise to some difference of opinion. Some authors (Agassiz and Whitman, '84; Ziegler, '87) regard the first three furrows in the two groups as equivalent. Others (Rauber, '83; H. V. Wilson, '91) believe that the first equatorial furrow in the frog has been "lost" in the Teleost. Our observations on Amphibian cleavage bear, we think, directly on this point. We have found that the third set of furrows, which is usually horizontal, may sometimes be truly vertical in one, two, three, or even all four quadrants. (See Figs. 2, 8, 26, 33, etc.) That in these cases there is any "loss" or "dropping out" of furrows is obviously absurd, especially since the third set, when thus irregular, appears simultaneously with the "normal equatorial" of other eggs from the same parent. Now, since the third set of furrows in the Amphibia is sometimes truly vertical, there is no occasion for surprise that the third set in the Teleost egg is normally vertical. To speak of any "loss" of the third set of furrows in Teleosts, seems not only vague but meaningless. It is not at all remarkable that the spindles, under the different conditions prevailing in the Teleostean egg should take up a position different from that taken in the Amphibian egg. In the Amphibian egg itself, as we have shown, they are free to take a variety of positions even at the third division.

All of our observations tend to emphasize the fact that great variation is a frequent occurrence in the early cleavage stages of the Amphibian egg. We have found irregularity to be the rule, regularity the exception. The appearance of the fourth set of furrows almost invariably marks the end of any constancy whatever in the relative position of the blastomeres. (See *e.g.*, Figs. 3, 4, 7, 13, 19, 24, 28, 32.) The interesting condition discovered by Roux ('83) in the eggs of *Rana esculenta*, which enabled him to distinguish right and left sides in the sixteen-cell stage, we have not met with in any of the eggs we have examined.

We can, furthermore, attach no great importance to the existence of the *Polflucht* as formulated by Rauber ('83). It is generally true, we admit, that the furrows do not pass through a common point on the upper pole of the egg, but occasionally they do (Fig. 15). We believe, consequently, that it is premature to formulate such a statement as that the furrows "alle suchen den Pol zu vermeiden." The furrows do not "avoid" the pole; but the mechanical cell-stresses are rarely so adjusted that the furrows intersect at the pole. There seems no need for a special term — *Polflucht* — to express this fact, since the "shunning" of the pole can hardly be a matter of primary significance.

Some of our observations bear indirectly upon the question of the relation of the first cleavage plane to the antero-posterior axis of the embryo. In the frog, as shown by the researches of Newport, Roux, and others, the first cleavage plane coincides with this axis; in *Diemyctylus* (Jordan, '93) and in *Triton* (Hertwig, '92), on the other hand, the first cleavage plane is at right angles to the axis. Our observations seem to indicate that this coincidence of the antero-posterior axis with one of the first two planes of division, is apparent rather than real. If such coincidence have any morphological meaning whatever, it must be in this way, that the derivatives of the cells on the right side of the first or second plane go to form the cells on the right side of the embryo. Our observations demonstrate, however, that the first and second cleavage planes undergo, even in the earliest stages, extensive torsion. Figs. 19,

20, 28, for example, show that cells originally to one side of the mid-line have been so shifted by the stresses of cell-division as to lie unmistakably on the opposite side. Everything indicates that the extent of this shifting increases greatly in later stages. It is, of course, open to any one to suppose that the cells thus driven out of their original position regain this position later on. The probabilities against such an occurrence, however, seem to us so great that we feel justified in considering that the burden of proof rests on those who would maintain that this readjustment takes place. It seems to us a more reasonable supposition that the direction of the early cleavage planes and the embryonic axes have no vital connection, and that the coincidence, where it exists, is in itself of no fundamental significance.

It seems to be unnecessary to dwell here upon the question of cell homologies. Reference to our figures will show abundantly that the derivatives of equivalent quadrants do not hold similar positions. There exists in this respect a fundamental difference between these vertebrates and certain invertebrate groups, notably the annelids. In the amphibia, the more detailed the study, the more frequent and radical appear the cleavage variations, while in the annelids, as Wilson ('92) expresses it, "the cleavage of the ovum takes place with a precision and regularity which oft-repeated study only renders more striking and wonderful."

THE UNIVERSITY OF CHICAGO,
March, 1893.

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PLATE XXVI.

In all the figures the continuous *black* lines represent the *first* cleavage plane, the *broken lines* the *second*, the *blue* the *third*, the *red* the *fourth*, the *dotted lines* the *fifth*. In most cases the precise time at which each furrow appeared is recorded on the figure.

FIGS. 1-10 represent eggs of *Rana*; Figs. 11-15, *Bufo*; Figs. 16-24, *Diemystylus*; Figs. 25-33, *Amblystoma*.

FIGS. 1-4 represent successive stages of one egg viewed from the upper pole.

FIGS. 5 and 10, respectively, top and bottom views of one egg.

FIGS. 6 and 7, successive stages of one egg from upper pole.

FIG. 8. Upper pole; fourth set of cleavage planes just appeared.

FIG. 9. Upper pole; fourth set of cleavage planes just appeared.

FIGS. 11-13. Successive stages of one egg; upper pole.

FIG. 14. Lower pole of egg shown in Fig. 13.

FIG. 15. Upper pole showing conformity to diagram type of cleavage.

FIGS. 16-19. Successive stages of cleavage of one egg showing remarkable irregularity. A normal embryo was formed from this egg!

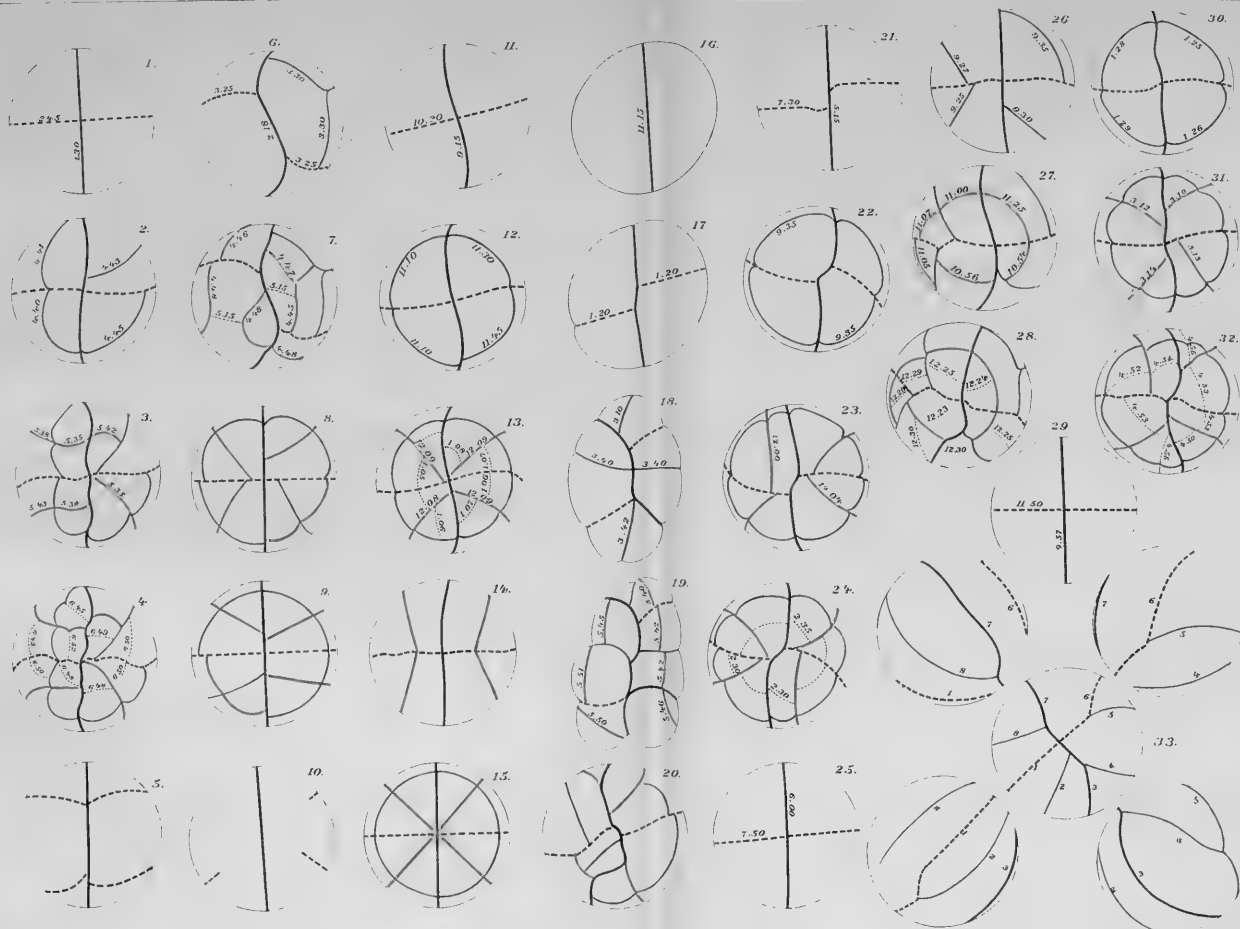
FIG. 20. Upper pole showing great distortion of first cleavage planes.

FIGS. 21-24. Successive stages of one egg; upper pole.

FIGS. 25-28. Successive stages of one egg; upper pole.

FIGS. 29-32. Successive stages of one egg; upper pole.

FIG. 33. The central figure represents the upper pole of the egg, the surrounding figures are side views of the same egg. To each furrow is affixed a recognition number.



NOTES ON REGENERATION AND HETEROMORPHOSIS OF TUBULARIAN HYDROIDS.

ELIZABETH E. BICKFORD.

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I. INTRODUCTION.

THE regenerative power of *Hydra* was first studied by Trembley in 1744. He observed the general appearance of the healing, and the subsequent regeneration of pieces, obtaining fully formed hydroids in a few days after they were cut. Nussbaum and Ischikawa (12) have since repeated and confirmed his experiments. They found, as did Trembley, that the character and position of the regenerated organ is predetermined in the uninjured animal, and that consequently tentacles always grow from the anterior end, and the foot from the posterior end of the cut portion. More recently, Jacques Loeb (10, 11) has confirmed these conclusions by experiments described fully in his work on "Heteromorphosis"; —he has shown that this law of predetermined orientation holds good not only for the *Hydra*, but for many other forms of animals with which he experimented. But he has also shown that there are animals in which, as in certain plants, it does not hold good; in these the position of the organs can be varied by external conditions. For example, he has shown that roots could be readily produced on oral ends of hydroid stems by simply bringing the regenerating end in

contact with a solid surface ;— on the other hand, the hydranths were produced on both ends of the stem when the water was allowed to play freely around the cut ends. To this phenomenon of the reproduction of an organ typically different from the one which had originally occupied that position, he has given the name heteromorphosis, and thus distinguishes it from the more usual phenomenon of regeneration through which a lost organ is replaced by one of the same kind.

The experiments to be described in the following paper will serve to confirm and illustrate this principle of heteromorphosis ;— they will also deal with the regenerative power of *Tubularia tenella*, and with some of the regenerative processes.

This work was suggested by Dr. Loeb, and was carried on at the Marine Biological Laboratory at Woods Holl. The hydroids were obtained from New Bedford, where they were found growing in great abundance during the early summer months. The size and transparency of the individual stems, also the fact that this form does not branch, rendered them especially suitable for such study ;— the last-mentioned characteristic aids in proving that the heteromorphosis of these forms is *genuine*, and does *not* depend upon any tendency to bud, as has been suggested by Trauttsch in opposition to Loeb's conclusions.

II. PROCESSES OF REGENERATION.

(a) In order to give a clear conception of the changes which occur in the processes of regeneration, a brief sketch of the general anatomy and histology of hydroid structure gleaned from descriptions by Allman (1), Hamann (5), Jickeli (7), Ciamician (3) and others, will be presented here. The Tubularian hydroid consists of a stem portion,— the hydrocaulus,—terminating at the aboral end in a root-like expansion,— the hydrorhiza,—and at the oral end with the hydranth. This stem portion, which was the part chiefly used in these experiments, contains the fleshy tube-like axis known as the coenosarc, which is surrounded by an external horny layer, or covering, the perisarc. The coenosarc is mainly

concerned in the regenerative processes, hence its anatomy and histology are of special interest in considering the questions presented by the phenomena of regeneration. The outer ectodermal layer consists mainly of large cells, among which are scattered smaller cnidoblasts; beneath these there is a layer of muscle cells.

Beneath this muscular layer is found the transparent "Stützlamelle," or supporting layer, which is strongly developed in the Tubularians.

Lastly, below this layer is found the endodermal layer, which consists of large cells possessing nuclei lying near the walls, imbedded in a finely granular protoplasm. These cells also usually contain red pigment granules;—it is owing to the presence of this pigment that the pink tint of the hydroids is due. On the hydranths the endoderm cells show considerable differentiation. According to Hamann (5) the cells of the oral part are quite different from those of the middle and basal portions. In the first-named region the cells are longer and more slender than are the cubical cells in the "stomach" portion;—secreting glandular cells are found in the hypostome portion which stain more intensely in carmine than do the other cells. The endoderm of tubularian hydranths presents a series of folds or "taeniolae," which are usually four or five in number in the hypostome region; these may branch to form eight or ten folds in the stomach region, while below they pass into the simple entodermic layer of the stem.

It is unnecessary, for the present purpose, to enter more deeply into the histological details of the cell structure. This review of hydroid structure will suffice to show that what at first glance appears to be a case of very simple regeneration, in the formation of new hydranths from stem portions, is in all probability a comparatively complex differentiation of stem tissues into those possessing the various functions of absorption, secretion, etc. This must be the case, if, as Allman says, we are justified in considering the portion of the somatic cavity included in the hydranth, as chiefly devoted to digestive processes. Before passing on to the experimental results, there remains to be described the somatic fluid which fills the

body cavity, and is readily seen circulating rapidly in the regenerating ends. Allman describes this as a transparent liquid, in which are the following solid bodies :—disintegrated elements of food, cells which have doubtless been detached from the walls, and minute irregular corpuscles, which are possibly effete elements. It is in the hydranth that the food is dissolved ; of the nature of this solvent we know but little. According to G. Greenwood's paper on "Digestion in Hydra," it doubtless originates as a secretion from the walls. The products of this digestion become the somatic fluid, which is mingled with water from outside, and propelled by the ciliated endodermal cells through the cavity of the coenosarc, in currents which are rarely definite. In developing buds these currents possess great activity ; in limited cavities they usually pass in circular streams as in the hydranths, being here most likely connected with the preparation of aliment. Whether this fluid conveys the fully formed pigment to the regenerating ends, taking it from other cells, or whether it is formed *in situ*, as a result of rapid growth and metabolism, is a point which remains to be determined by more minute histological study.

This point is of interest in considering the question as to the source of the material used during the extremely rapid growth and regeneration of the hydroids studied. In many instances fully formed hydranths were obtained *in about eighteen* hours after the old ones were cut off. The comparatively large size of some of the hydranths regenerated on very short fragments indicated that the supply of reserve material, in some form, must be abundant. The amount of pigment in the regenerating ends would lead one to suspect that there may be a connection between it and this reserve supply ;—this also is a point worthy of histological investigation.

(b) The experiments were made with these hydroids in the following manner :—Fresh stems of hydroids were taken from colonies, the hydranths cut off, and the stems cut in pieces of varying sizes. These were placed in a bowl of fresh sea water and covered with glass to exclude the dust. Some extremely short pieces were examined under a high power

immediately after cutting, by placing them on one end, under a coverslip, in a cell-slide, and providing them with water enough to cover fully. The interior could thus be easily studied. Each fragment appeared at first as a simple ring (see Fig. 1), in some cases with a system of endodermal cells across the middle—open freely at both ends; no signs of any somatic fluid were visible at first; in about five minutes the peripheral cells of the cut ends were seen to elongate gradually (Fig. 2) and to extend in an amoeboid manner towards the center; this continued until they met in the center, thus closing in the cut ends entirely. The cells at this point presented a distinctly elongated radiate arrangement (Fig. 3). The membrane thus formed over the cut ends was very thin and allowed the vigorous circulation of somatic fluid (which appeared very soon after the closing) to be readily seen. In an hour's time this circulation had become very rapid, and many masses which looked like cells, and pigment granules were seen whirling about in the central cavity.

In order to observe these first stages of healing more fully, fresh stems were taken, placed under the microscope and longitudinal fissures made in them with needles. At first a slight gaping of the wound appeared; very soon, however, the cells along the edges elongated towards each other until in a very short time the wound was completely closed, and circulation of the fluid, which had apparently been inhibited by the wounding, was again established. In all cases the healing was very rapid. Evidently both ectoderm and endoderm had taken part in this process as the wounded ends of the coenosarc (seen in Fig. 4), when viewed from the side, showed the pigmented layer beneath the colorless ectodermal layer.

After the healing of the cut ends no changes were visible for some time; the membrane at the ends of the coenosarc remained thinner, and the somatic cavity larger. Whether this was due to the distension of cells in that part, or an actual thinning of the walls, owing to the wandering away of some of the endodermal cells into the central cavity, was not determined. The rapid circulation and next visible change in the regenerating portions indicated that some changes in the

nature and arrangement of the cells was taking place. The whole end became more deeply pigmented, — which may have been due to the increase of endodermal cells forming the folds of the hypostomal region — and two rows of pigmented spots appeared ; one near the end, the other a short distance below. This lower row soon became elongated to pigment bands, see Fig. 4a, which were later found to be the tentacles. Allman figures a stage similar to this in his work. Up to this period all changes had taken place within the perisarc, with no visible increase in the size or length of the regenerating coenosarc. This appears to me to be a point of much interest, as it apparently indicates that a large part of the changes in the formation of new hydranths are not alone due to increase in bulk by new growth, *i.e.* a development from a *few* indifferent cells, as Lang describes for budding hydroids, nor to the restoration of each part, as Weismann says occurs in the regenerating hydra. (In the case of this hydroid at least, the regeneration appears to be largely a direct transformation of a stem portion over into the body portion of the new hydranth.) This may imply that the endoderm cells of the stem portion have in some way undergone a differentiation or transformation, fitting them more definitely for the various functions of the digestive processes, which take place in the hydranth. Since finishing the experiments described here, I have been unable to make out these histological details of the cell-changes of this form, for lack of material. I have, however, investigated this point in my work on *Cordylophora* and have been able to obtain sections showing different stages in the transformation of the more cubical endodermal cells of the stem, into the more elongated cells of the regenerating hydranths.

It is worth while to notice it was observed, in connection with this work, that regeneration does not appear to start from a *few undifferentiated cells*, as in the case of budding in hydroids, nor from *one* cell, as in the case of embryonic development, but that *all the cells in the regenerating end take part directly in this process*. What is still more suggestive is that this fact admits of the interpretation that possibly the histological and physiological qualities of the cells of an adult tissue

are by no means as absolutely fixed and unchangeable as some of the modern ideas of heredity make it appear.

The next stage in the regenerative process is shown in Fig. 5. After the formation of the tentacles, with their central axes of richly pigmented cells, the hydranth emerges from the perisarc, and from this time on the growth of the stems in length is quite rapid, in some cases as much as 7 mm. in six days.

(c) Another experiment, showing that the *complete* hydranth is formed within the perisarc, was performed as follows:—A large stem attracted special attention by the arrangement of the pigmented bands, which appeared rather farther apart than usual. In order to study this more fully, ten sections of the stem were made, one below each pigmented row (see Fig. A, x and y). The contents of each piece were carefully pressed out of the perisarc containing it.

The first piece was found to be an irregular mass, with a fringe of very short tentacles around one side (see Fig. 7). Shortly after it was pressed out, the cut ends closed in, and it assumed a more hydranth-like form (see Fig. 8). The second fragment was then examined and appeared somewhat hydranth-shaped, with a large proboscis portion, surrounded by a row of long tentacles, which were in slow motion (see Fig. 10). Evidently the first cut had been made between the regions of the oral and coronal tentacles, dividing the hydranths into two parts. These fragments were placed in fresh sea water until the next morning, when they were again examined. The first portion had become definitely hydranth-shaped, and the tentacles had increased in length (see Fig. 9). The second fragment had also changed (Fig. 11), the proboscis was still much larger than in the normal hydranths. This form was also in very active motion. The next day both fragments were dead.

III. REGENERATION OF TRANSVERSE SECTIONS OF TUBULARIA.

(a) While studying the process of regeneration a small stem about 3 mm. in length was found, on which was the old hydranth, not completely severed, yet alive, and a new

hydranth on the oral end; both were in vigorous motion (Fig. 12, a, s, b). A second cut was made about half way down the stem, and the next morning a new hydranth (Fig. 12, c) was found at the oral end of the lower half. All three hydranths were in vigorous motion.

The ready regeneration of this small piece seemed to indicate that the power depended but slightly upon the size of the fragment; to study this point further the following experiment was made:—

(b) Seven whole stems were placed in the first bowl, ten half stems in the second, twelve fourth stems in the third, and twenty-four eighth stems in the fourth bowl. After two days the following results were observed: The first stems had regenerated four hydranths; the second series three hydranths; the third series ten, and the fourth series twelve hydranths. These were allowed to grow for five days. Then the increase in length was measured, and found to be as follows: The whole stems averaged 7 mm.; the half stems 6.5 mm.; the fourth stems 5.5 mm., and the eighth stems 4 mm. Thus the actual amount of growth was proportionally much greater in the stems when cut into eighths than in the whole stems.

(c) While working on the pieces of stems some of the regenerating fragments were so small that the question presented itself as to whether there were any *special regenerating* regions, comparable to budding zones, or to specialized regions of reproductive cells, as Weismann describes in his paper on Sexual cells of Hydro-medusae (14). Dr. Loeb had shown in his experiments that when stems were cut in halves the hydranths were formed at the same time on the oral ends of the fragments. An experiment was made varying from his in the number of sections of a stem. In this case a strong vigorous stem about 4 cm. long was cut into twenty sections; when examined later, sixteen of these fragments were found with complete hydranths. The four small pieces which had apparently not regenerated, were studied under a low magnifying power, and were found to contain abnormal hydranth forms. In experiments previous to this, numerous cases of heteromorphosis had been found, as nearly all fragments regenerated

a hydranth at each end. But in these four fragments this heteromorphosis appeared more marked, as they were so short that the whole of the stem had been transformed into two hydranths, which were thus united without any stem portion between. When these double-headed forms were pressed out of the perisarc they presented various abnormalities; in one case (Figs. 13 and 14) oral tentacles were found on each end, while in the center irregular-shaped tentacular buds were found; while in another case (Figs. 18 and 19) the two complete hydranths were joined directly; a flow of the somatic fluid from one to the other was readily seen. All these forms were in a vigorous state of activity; this was possibly one reason why they had not emerged from the perisarc in the usual way. This experiment showed conclusively that the regenerative power was *not* limited to any particular regions of the stem, and at the same time it suggested the following experiment, in order to determine the limits of the regenerative power:

(d) A second stem about 4 cm. was cut into *fifty* transverse sections, which were placed in water as usual. After two days they were examined, with the following interesting results: Twenty pieces were found with fully formed hydranths on one end; one with hydranths on each end; twenty other fragments showed abnormal regenerative forms, which had not emerged from the perisarc. A few typical abnormal forms may be described here. One was found (Figs. 15 and 16) which had the usual oral tentacles while coronal tentacles were lacking; and in their place, at the other end, were found tentacle-like buds, and two fully developed tentacles projecting aborally. Another form (Fig. 17) was double-headed, but the number of coronal tentacles was complete on each hydranth. Still another form, found in a very small section, appeared to possess no definite form (Figs. 20-21), but was a rounded mass, with a row of knob-like tentacles on either side; here evidently the stem was not long enough for even one hydranth. It was, however, in vigorous motion. In one case (Fig. e, 2) a very small fragment had become freed from the sides of the perisarc, and showed great contractile power while in it.

These experiments showed that if there are any formative cells upon which the initiation of these regenerating processes depend, they are not restricted to any special regions, — whenever a transverse section was made, a tendency to form hydranths was found. At the same time these experiments tend to show that the regeneration is more a process of transformation than new growth, as, where the stem portion to be transformed was too limited, abnormal forms often result, instead of a *growth* with a complete hydranth. From the last experiment described, the conclusion may be drawn, that the tendency to form *one* complete hydranth of short pieces is rather more marked, than to form double abnormal forms.

(*c*) Later in the season, when most of the colonies were found to have lost their hydranths, experiments were made with stems and roots, to ascertain if under these conditions of a normal resting stage there was any concentration of regenerative material in the roots. Twenty-two pieces of roots and twenty-six pieces of stem were placed in sea water. The lengths of the stems varied from 7 to 14 mm., while the pieces of roots varied from 1 to 4 mm. When these were examined, on the fragments of roots were found thirty-two hydranths; when these branch, as only the roots do in this form, hydranths were found on each branch, thus illustrating beautifully the principle of heteromorphosis.

The number of hydranths on the stems was only thirteen. The hydranths on the roots were much smaller than those on the stems, which follows naturally, as the diameter of the coenosarc determines the size of the hydranth. After several days the growth of the roots and stems was measured. The average increase in length of the former was from 4 to 5 mm., while the stems had grown only from 2 to 3 mm. As these experiments were not made when the colonies were in their most flourishing condition, it cannot be stated whether these results would hold good for *all* conditions, or whether they indicate a concentration of growing material only during a resting period.

IV. REGENERATION OF LONGITUDINAL SECTIONS OF TUBULARIA.

It remained to be seen whether results similar to those described, could also be obtained from longitudinal sections.

Trembley experimented upon *Hydra*, cutting them in halves lengthwise; he stated that healing occurs so rapidly that in three hours they were able to eat as usual. In this case there was evidently only a closing of the wound, and not a formation of new parts. The experiments to determine the ability of split portions of the coenosarc to form hydranths, were made as follows:—

Several good-sized stems were cut open longitudinally; some of the fragments thus obtained were very small and narrow, other stems were only partly split. These were examined the next day, and all the portions of coenosarc, even the smallest, were healed and rounded in form; they showed a most vigorous circulation within (see Fig. 23). When examined later, most of these portions had not regenerated; one stem was found, however, which presented the following interesting conditions (see Fig. 24): it had been split only half way down, the lower portion of the stem remaining whole. On the two split portions of the perisarc there were four separate rounded masses of coenosarc, each of which had regenerated, two with hydranths directed aborally and two orally, while from the point of union of the two, a fifth good-sized hydranth had appeared. As usual, the sizes of these hydranths were proportional to the diameters of the masses from which they were formed.

From these experiments it may be concluded that, while split portions may regenerate, yet this does not take place as readily as is the case with transverse sections. In all cases they pass through the first stages of regeneration, healing very readily. The fact that they are unprotected by the perisarc, and thus left exposed to the action of the water, may account, in part, for their failure to regenerate completely.

CONCLUSION.

The chief results of this paper, briefly stated, are as follows:—

1. The regeneration of the hydranths of these Tubularians, is due, *not* to simple processes of *budding or an entirely new formation*, but largely to the transformation of the tissues of the stem, into the body portion of the hydranth.

2. That where the portions of stem are sufficiently short, the *whole coenosarc may be transformed into the two hydranths, with no stem portion intervening*; or, in some cases, it may be transformed into one hydranth; while in other cases, where the portion of the stem is still shorter, it may be transformed into a partial hydranth, *which is not completed by any process of growth*.

Since completing these experiments at Woods Holl, I have had the opportunity, while doing some work in the Johns Hopkins Laboratory, of repeating some of them upon the *Cordylophora*, which was easily obtained in Baltimore. I found this form possesses similar regenerative power; stems often being obtained which showed the heteromorphic formation of hydranths on both ends of stems.

The regenerative processes appeared to differ in some points from those found in *Tubularia tenella*. I hope to determine more fully these regenerative processes for both forms (the *Cordylophora* and *Tubularia*) in a future work, as the results of a careful investigation of the histological changes which take place during the transformation and growth of stem portions into hydranths cannot fail to be of much interest when compared with the processes of budding and embryonic development.

MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASS., 1892.

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EXPLANATION OF FIGURES.

(These sketches were made from living forms, studied under a low magnifying power.)

FIGS. 1, 2, 3. Regenerative process. Fig. 1, healing of the cut end in the first stage, *i.e.* when first cut. The thin ring of coenosarc, with its central septum, is surrounded by the perisarc; Fig. 2, same a few minutes later; the ends of the cells have begun to elongate towards the center; Fig. 3, the same later; the cells have met in the center, completely closing the wound. (These three figures are diagrammatic.)

FIGS. 4, 4a, 5. Hydranth formation. Fig. 4, stem as it appeared two hours after healing, showing enlarged somatic cavity at one end; Fig. 4a, same several hours later, showing the thickened pigmented spots at the upper end, and bands of deeply pigmented cells below; Fig. 5, same later, with hydranth just emerging from the perisarc.

FIGS. 6, 7, 8, 9, 10, 11. Stem which was deeply pigmented in two places. Cuts were made at *x* and *y*, and the contents of each section pressed out; Fig. 7, contents of upper portion when first pressed out; Fig. 8, same shortly after; Fig. 9, same the next morning; Fig. 10, contents of lower section when first pressed out; Fig. 11, same the next morning.

FIG. 12. Portion of stem 3 mm. long. *a*, Old hydranth not completely severed from the perisarc; *b*, new hydranth seen the next day; *c*, third hydranth found the following day.

FIGS. 13, 14, 18, 19. Parts of a stem cut in twenty parts. Fig. 13, form as seen in the perisarc; Fig. 14, same pressed out; Fig. 18, form seen in the perisarc; Fig. 19, same when pressed out.

FIGS. 15, 16, 17, 20, 21, 22. Forms regenerated from portions of a stem cut into fifty parts. Figs. 15, 17, 20, 22, are forms within the perisarc; Figs. 16 and 21, forms are same as 15 and 20, only pressed out of the perisarc.

FIGS. 23, 24. Regeneration of split stems. Fig. 23, small fragment of split perisarc, upon which are two small portions of the coenosarc; these have healed and showed rapid circulation in even the narrowest part; Fig. 24, split stem with five complete hydranths; four of these from fragments of coenosarc, and one from the main stem. One small portion of the coenosarc has not fully regenerated.



THE NERVOUS MECHANISM OF THE RESPIRATORY MOVEMENTS IN LIMULUS POLYPHEMUS.

MISS IDA H. HYDE.

THE following paper gives the results of experiments on the nervous mechanism of the respiratory movements in *Limulus*, which were made under the instruction of Dr. Jacques Loeb during the summer of 1892 in the Marine Biological Laboratory at Woods Holl.

Very few investigators have experimented to determine the influence of the nervous system on the respiration of invertebrates. Of these, Faivre's work on *Dytiscus* (iv) is the most important. He found that in *Dytiscus* the respiratory mechanism is controlled by three centres: (*a*) the metathoracic ganglion, which presides over respiration, (*b*) the suboesophageal ganglion, which coördinates respiratory with post-abdominal movements during walking or swimming, and (*c*) the abdominal ganglionic cord, serving as a conducting organ for the centres.

Limulus is in every respect a more favorable subject to experiment on than is *Dytiscus* or any other invertebrate that I know of. Its power of endurance is unequaled, and its large size enables one to operate on it with great certainty and ease.

The object of the experiments was to determine by which region of the nervous system and in what way the mechanism of respiration was controlled.

The abdominal ganglionic cord in *Limulus* (*D* Fig. 1), as we shall see, is the only part of the nervous system concerned in the respiratory movements. Therefore, it alone requires description. The double nerve cord is inclosed in an artery and extends along the ventral side of the abdominal region to the arms. It contains six ganglia, from each of which arise two pairs of nerves. The first five larger or posterior pairs of

nerves supply the first five gill-plates, whilst the sixth pair goes to the anal region. The anterior pairs of nerves go to the integument and give off fibres that run forward and unite, forming on each side a lateral longitudinal nerve. These nerves are known as the lateral nerve chains.

The respiratory movements in *Limulus* consist in a consecutive dropping and raising of the gill-plates, respectively known as the inspiratory and expiratory phases. The former consists of a rhythmical dropping of all the gill-plates from the ventral abdominal wall; at the same time the gill-books (Fig. 4) become fully dilated and their contents of blood thoroughly exposed to the water. The expiratory phase consists, first, of a quick, consecutive, upward beat of the gill-plates against each other and the ventral wall; second, a slight forward pressing of the posterior margins of the plates. During these stages the gill-books are first flattened, then slightly compressed against each other, and at the same time their contents are forced out.

A normal *Limulus* makes on an average twenty-seven inspiratory movements a minute, when the temperature of the water is about 24° C. I often noticed that when normal *Limuli* lie motionless the movements cease for an hour or more; that is, the gill-plates remain in the expiratory phase, then, for no apparent cause begin spontaneously to move in their regular rhythm. Moreover, in normal *Limuli*, occasionally, and always after an operation of some part of the nervous system, one can see a peculiar, respiratory activity or cramp-like movement of the gill-plates. A better understanding of this is gained from Fig. 3, which gives the position of the plates during the movement.

I. LOCALIZATION OF THE CENTRE OF RESPIRATORY MOVEMENTS.

A. Experiments on the Brain.

The investigation was begun by performing various operations on the brain and noting their effects. It may be said here that the results of these experiments warrant the conclusion, that lesion or loss of part or the whole brain (anterior

oesophageal ganglion) does not influence the respiratory mechanism of *Limulus*. For if we cut either transversely or longitudinally into the brain (*B* Fig. 1), — or, if we injure or extirpate half, more or less than half, or even the entire brain, we find that when the effects of the operation have passed off, the respiratory movements still remain normal.

B. Experiments on the Oesophageal Collar.

Of the results of the experiments on the oesophageal collar, it need only be said, that they had no influence on the respiratory movements. If we cut between the ganglia of either side of the collar, extirpate one or more of the ganglia (*H* Fig. 1) or remove the entire collar (*C* Fig. 1), the respiratory activity still continues.

C. Experiments on the Post (sub) Oesophageal Ganglion.

The results of Faivre's experiments led me to think, that the post-oesophageal ganglion might influence the respiratory activity of *Limulus*. If, however, we injure or extirpate part or the whole of the ganglion (*G* Fig. 1), we find that the respiratory movements still persist in their normal activity.

For several days after the operation, the gill-plates may remain, sometimes for hours, in the expiratory phase, while the animal remains perfectly quiet. But after a while the respiratory activity spontaneously begins again, and continues for days and even months. The interrupted respiratory movement is not a matter of consequence, since, as was said, it often occurs in normal *Limuli*.

D. Experiments Anterior to the Abdominal Cord.

Perhaps the most difficult and interesting operations were those, in which the whole brain, with the oesophageal collar and post-oesophageal ganglion were extirpated, thus leaving only the abdominal cord (*D* Fig. 1), which now had an opportunity to prove its function, unaided by any other part of the central nervous system.

During such an operation the animal loses much blood and is left very weak, and since it can no longer swallow food, it cannot be strengthened.

Immediately following the operation, the respiratory acts may stop for an hour or more. A touch, however, on either the gill-plates or carapace will arouse the activity for a short interval. After a few hours the plates begin to move spontaneously, and only occasionally is their rhythm interrupted by cramp or cessation of movement.

One animal lived three days after the operation, and was then subjected to the post-mortem.

In another *Limulus* the whole brain, post-oesophageal ganglion, and all but three ganglia of the collar were extirpated. The three ganglia were left, so that the animal could be fed and kept alive. To my satisfaction, the animal lived three weeks after the operation. During all of that time the respiratory activity continued quite normally; at times there were intervals of cramp or interrupted respiratory movements, but after a while, the gill-plates spontaneously began their regular rhythm again.

The experiments have therefore proved, beyond all doubt, that the respiratory movements of *Limulus* can continue when only the abdominal cord is left; furthermore, that the centre of the respiratory movements is not located either in the brain, oesophageal collar, or post-oesophageal ganglion.

E. Experiments on the Abdominal Cord and its Ganglia.

(a) The abdominal cord was cut through, just anterior to the operculum (*O* Fig. 1). Thus the connection between the brain and the cord is destroyed. In such an animal a few hours after the operation, periods of about seventy-five seconds of slow respiratory movements alternate with periods of about sixty seconds of complete cessation.

The day following the operation, intervals of slow respiration alternate with intervals of cramp movements, as the following observations indicate:—

10.10	A. M.	22	inspirations in 60 secs.,	cramp movement, 60 secs.
10.12	"	30	" " 90 "	" " 75 "
10.14 $\frac{3}{4}$	"	25	" " 60 "	" " 75 "
10.16	"	13	" " 30 "	" " 45 "
10.17 $\frac{1}{4}$	"	33	" " 90 "	" " 60 "
10.19 $\frac{3}{4}$	"	34	" " 90 "	" " 75 "

During the next two weeks the respiratory activity did not vary much.

(b) The abdominal cord was cut transversely between the operculum and the first ganglion (*I* Fig. 1). In some *Limuli* the mechanism did not differ from that of the above. In others the respiratory movements were very slow immediately after the operation. The following day they became intermittent, that is, they alternately ceased for a time, then returned at times above, at other times below the normal, and occasionally cramp movements appeared. Several days later, as the animal weakened, the respiration gradually ceased.

The gill-plates all move in the same rate, extent, and rhythm, and have cramp or interrupted respiratory movements at the same time.

(c) When only the artery that ensheathes the cord is cut, and not the nerve, allowing a loss of much blood, with the exception of short periods of cramp or no movements, the respiratory mechanism of *Limulus* is not altered.

(d) In another series of experiments, the cord was cut between the second and third ganglia (*II* and *III*, Fig. 1). Thus the gill-plates were divided into an anterior division of two plates, still connected with the brain, and a posterior division of three plates separated from it. In some cases, for the greater part of the time the anterior division moved quite normally. The posterior division part of the time made slow movements that alternated with cramp movements, about as noted below :—

4.45	P. M.	17	inspirations in 35 secs.,	cramp movement, 60 secs.
4.46	"	22	" " 50 "	" " 60 "
4.48 $\frac{1}{2}$	"	26	" " 55 "	" " 55 "

Two weeks after the operation, the anterior and posterior division moved in unlike rhythm, and at times cramp or interrupted movements appeared for short intervals in the posterior division.

(*e*) The cord was cut both anterior to the first and third ganglia (I and III, Fig. 1). The gill-plates are thus divided into two divisions, both of which are separated from the brain. The first and third ganglia were affected by the operation, and the corresponding gill-plates move only passively. Often the first two plates have cramp movements, while the others continue at their usual rate. Observations made during the two weeks following the operation showed that the first division moved in a rhythm differing from that of the other, and was not affected by cramp or interrupted respiratory movements of the latter. Moreover, the posterior was not influenced by the behavior of the anterior division.

Experiments (*d*) and (*e*) demonstrate, that when the gill-plates are divided into two divisions, one or both of which are separated from the brain, each division has its own peculiar rhythm, and is not influenced by the behavior of the other. We can conclude, therefore, that each has its own rhythmical centres.

(*f*) The second ganglion was extirpated (II, Fig. 1). By its removal, the second gill-plate lost its sensibility and motility. It is moved by the action of the water and neighboring plates. All the other plates continue their activity. If we hold the second plate away from the others, we see that each division continues to move in its own peculiar rhythm and extent.

During the two months that observations were made, no marked change appeared in the respiratory movements. At times the posterior division was either motionless or in cramp movements, while the anterior behaved normally. At other times the latter had cramp movements, while the former did not, and usually the two divisions moved in different rhythms. When the second ganglion is cut through transversely the results are practically the same as those above. The same results were obtained with other *Limuli*. This experiment also showed that destruction of the ganglion at once arrests the

activity of the corresponding gill-plate ; and, provided that the ganglia of the cord are left intact, while the remainder of the central nervous system is destroyed, the respiratory movements are not abolished. This of itself is enough to show that the ganglia of the cord include the chief centres of respiratory movements.

(g) The fourth ganglion (consisting in the adult of the fourth, fifth, and sixth united) was cut through longitudinally, so that the nerves of the left and right sides were left attached to their respective halves. Several hours after the operation, although both sides of the fourth and fifth plates began inspiratory activity together, the left moved more anteriorly and with greater extent than did the right sides, and in the expiratory phase they did not move so far posteriorly. Occasionally the left sides had cramp movements, while the right were moving normally, and this was often followed by cramp movements of the right sides, during which the left sides moved on uninterruptedly. Besides these many other observations were made on Limuli operated in this way. They strengthened the conclusion that each half of a ganglion controls, to a certain extent, the function of its own side.

F. Experiments on the Peripheral Nerves.

The following experiments were made to ascertain the function of the anterior and posterior nerves. As was said before, the anterior nerves send fibres to the integument, and the posterior to the gill-plates.

(a) The posterior nerve was exposed, ligatured in two places, and cut between the ligatures.

Each time that the distal end was stimulated with induction currents of moderate intensity, that half of the gill-plate to which the nerve was attached made inspiratory movements. Neither the appendages, abdominal carapace, nor other plates moved. Therefore, the posterior nerve contains motor fibres.

(b) When the electrode was applied to the proximal end of

the posterior nerve, the appendages beat about in all directions. Therefore, the posterior nerve contains also sensory fibres.

(c) The anterior nerve was exposed, ligatured, and cut ; no movement occurred when the electrode was applied to the distal end. But each time that it was applied to the proximal end, the appendages beat about and the carapace moved. The anterior nerves contain consequently only sensory fibres.

(d) The abdominal cord was cut, and when the posterior cut end was stimulated with the electrode, the gill-plates posterior to the cut made inspiratory movements.

G. Experiments on the Lateral Nerve Chains.

The lateral nerve chains, as was said, extend on each side of the abdominal cord. They receive fibres from the anterior nerves, and are considered, by some authors, to be the Sympathetic System.

Both the right and left chains were exposed, ligatured, and cut, and the proximal and distal ends stimulated electrically, mechanically, and chemically. In every case the stimulus produced no apparent effect. Several hours after the operation the respiratory movements were quite normal. The lateral chains exercise, therefore, no influence over the respiratory movements.

II. REGULATION OF THE RESPIRATORY MECHANISM.

We know that the respiration of higher animals is influenced, among other causes, by muscular activity, sensory stimuli, and the condition of the medium surrounding them.

It became of interest to know whether the respiratory movements of *Limulus* were also affected by these. For this purpose several experiments were made, of which the following are the chief ones:

A. Variations of Respiratory Activity.

(a) The following experiment was made to ascertain if muscular activity had an influence on the respiratory acts of *Limulus*. If the second ganglion of the cord is extirpated, it

will be remembered the plates are divided into two divisions: an anterior of one plate connected with the brain, and a posterior of three plates separated from it.

By laying sticks across the appendages of such an animal it is forced to exercise its muscles. For, in endeavoring to remove the annoyance, it continually strikes against the sticks with its appendages and with increasing efforts.

Before the exercise both divisions made on an average twenty-three inspirations a minute. After an hour's exercise the anterior as well as the posterior division made on an average twenty-seven a minute, showing that exercise increased the respiratory activity of each plate, and to the same extent.

Other Limuli were treated in the same way and with the same result.

(b) It was of special interest to see whether a nervous stimulus influenced the rate and extent of the plates alike, when they were divided into two divisions, by lesion between the second and third ganglion.

When the animal was quiet one person timed the inspiratory acts of the anterior, at the same time another noted those of the posterior division. The result was that for every observation the posterior and anterior made about an equal number of inspirations in the same time and with the same extent. This was repeated several times with like result.

After that, the different appendages were pulled, so that the *Limulus* became greatly irritated. The plates anterior to the lesion made exaggerated inspiratory movements, just as all the plates of a normal *Limulus* would if thus treated. On the other hand, the plates posterior to the lesion but slightly increased their extent.

In one case the anterior made thirty-one, while the posterior division only made twenty-one inspirations during the same space of time. The result was the same with slight differences each time that the observation was made.

This might have been expected, because the anterior division being connected with the central nervous system would be affected by every cerebral impulse, while the posterior, which is separated from it, could not be so influenced.

(c) When *Limulus* masticates and swallows food, respiratory activity that has been temporarily stopped will immediately be aroused. This is true, not only in a decapitated *Limulus*, but also in one that had the entire brain, post-oesophageal ganglion, and all but three ganglia of the collar extirpated.

If food is swallowed by a *Limulus* whose gill-plates were divided into two divisions by lesion, both divisions increase in rate. If the anterior is moving normally and the posterior is motionless, the anterior moves with greater extent, and the posterior immediately begins respiratory activity.

The above facts show that it is not cerebral activity that influences respiration at the time of feeding. It may be the gastric and intestinal movements that reflectorally affect the respiratory mechanism.

When a *Limulus* is in a dying condition its respiratory activity is greatly altered. In one *Limulus* that had the cord cut anterior to the operculum (*O* Fig. 1) the respiration was characterized by intervals of rapid and exaggerated inspirations that gradually lessened and were then followed by periods of cramp movements. In another striking case the respiration of the last day was marked by movements that ultimately declined to complete cessation and returned to an amplitude much above the normal; resembling, to a certain extent, the Cheyne-Stokes respiration of higher animals.

B. Reflex Actions.

(a) If we apply external stimuli to a normal *Limulus*, its respiratory movements can be altered, and it behaves in a peculiar manner.

When it is placed on its back, and its spine, appendages, gill-plates, or any part of the ventral carapace is pressed with fingers or forceps, the gill-plates stop in the expiratory phase about as long as the pressure lasts (several minutes), at the same time the animal usually turns onto its ventral side; or it turns its abnormal carapace ventrally, the spine forward and pulls the appendages into the cavity of the cephalo-thorax.

(*b*) Nearly the same result is obtained in those Limuli that had part or whole of the brain, collar, or post-oesophageal ganglion removed (Fig. 1).

(*c*) In one Limulus in which the entire brain, collar, and post-oesophageal ganglion were extirpated, a very interesting result was obtained.

When the animal was in a dying condition, and the respiration had to all appearances ceased, it was found that they could be aroused again, and always for a certain period of time, by a slight stimulus on the plates or carapace.

For example, when the plates were motionless, and the abdominal carapace was pinched with a certain pressure, after a definite interval the plates began their activity, at a certain rate and extent. This activity lasted just seventy-five seconds, for a given pressure. If after a few minutes the stimulus was repeated, we obtained about the same number of inspirations in the same time. If the pressure was increased, the rate and its duration was also. This relation of stimulus and duration of reflex action was maintained for two hours, when a greater stimulus was required to arouse activity. The movements then lasted only sixty seconds, and the interval between pressure and reaction was lengthened. After an hour the activity of the plates could no longer be aroused.

(*d*) If, in a Limulus that had the cord cut from the post-oesophageal ganglion (*D* and *G*, Fig. 1), parts posterior to the lesion are touched, the gill-plates stand in expiratory phase for several seconds. But no sign of annoyance is evinced, either by the appendages beating about, or attempts made to turn over. If, however, parts anterior to the lesion are touched, so that the abdominal part does not turn ventrally, and thus have a secondary influence, the gill-plates are not disturbed in their rhythm.

(*e*) The following are some of the results on reflex action in Limuli, whose cord was cut between the second and third ganglion (II and III, Fig. 1). If parts posterior to the lesion are touched, the posterior division of plates stands in expiratory phase while the pressure lasts.

On the other hand, the regular rate of the plates anterior to the lesion, is not in the least altered by the stimulus. If, however, parts anterior to the lesion are touched, the appendages strike out in all directions and the anterior plates stop in expiratory phase. The posterior division is not interrupted in its rhythm, unless the abdominal part exercises a secondary influence, by turning ventrally.

(*f*) When the cord was cut, both anterior to the first and third ganglion (I and III, Fig. 1), both divisions of plates were separated from the brain, and, as was said, the first and third ganglia were injured by the operation. If one of the plates of the posterior division was pressed, both plates stopped a few minutes; then began rapid inspiratory movements; but the other plates were not disturbed in their rhythms.

When the second plate was pressed, it stopped in expiratory phase, several seconds, but no other plate was in the slightest influenced by the stimulus.

Pinching either the first or third plate produced no change whatever on the animal. Evidently they lost all sensibility.

Furthermore, no annoyance was exhibited, as, for instance, by beating about of appendages, if any one of the gill-plates was pinched.

The above observations have demonstrated that stimuli on parts posterior to the lesion have no effect on plates anterior to it, and *vice versa*. Each division of plates has its own reflex centres. When a ganglion was extirpated or injured, the reflex action of its corresponding plate was abolished.

Therefore, in the performance of the typical reflex actions above cited, the ganglia of the cord are the centres.

To conclude: The centres of respiratory movements, as has been shown, are situated in the ganglia of the abdominal cord. Their mode of action may be characterized as reflex and automatic: reflex, inasmuch as it may be temporarily increased or diminished by occasional peripheral impulses; automatic, as it seems to go on of itself, being kept going, however, by continuous stimuli that reach the respiratory centres, by the blood and the nerves.

If no outside stimulus is exercised, the rate of the plates,

even when divided into two divisions by lesion, is the same. The rate may be changed by exercise, but the change will still be the same for both divisions.

On the other hand, the extent and rhythm of the different divisions of plates is different, because the division connected with the brain is influenced by the latter, while the posterior is not.

III. GENERAL OBSERVATIONS.

In studying the behavior of the Limuli that had been operated, many peculiarities were observed, of which it was thought worth while to mention the following:—

(a) If we extirpate half of the brain of *Limulus* (*B* Fig. 1), after several hours we find that it has lost some of its normal habits. When it is placed in the sand it no longer burrows in it, nor does it turn on to its ventral side, or turn its spine forward when irritated while on its back.

Furthermore, its appendages are asymmetrically bent on themselves, on the side opposite to the one operated (Fig. 2). That is, they are bent on themselves so as to make a small angle between the third and fourth segment. The appendages on the side operated are not changed. As a consequence, the animal now moves in a circle toward the uninjured side. This movement, as well as the inability to turn on its ventral side, lasts *about two months, then gradually passes off*.

(b) If more than half of the brain is removed, the results are about the same as those above, with the exception that the gill-plates as well as the appendages of one side were asymmetrically held, with relation to the side of the brain most injured. The animal had forced movements to that side of the brain left uninjured, and at rare intervals one side of the gill-plates was inactive. At the end of three months the so-called forced *movements still persisted*. More than three months after the operation, *Limulus* was chloroformed and dissected, when an astonishing fact was discovered. It was seen that nerves which ran to the anterior integument from the brain, and that were cut in the operation, had regenerated and grown to a bit of nervous tissue left attached to the collar.

(c) Limuli that had the entire brain (*B* Fig. 1, anterior oesophageal ganglion) extirpated, are still able to masticate and swallow food that is given them. In most other respects they are greatly altered. Voluntarily, they never change either their place or position, and without some kind of a stimulus remain motionless in the most abnormal positions. For instance, if placed against the side of an aquarium, so that the anterior margin of their carapace is down and the spine up; or if their sides are down and the spine horizontal, they remain so, apparently comfortable.

A *Limulus* that has had its brain removed, makes definite and appropriate efforts to remove an irritant applied to any part. If it fails with one appendage, it makes attempts with others. Its movements result from the very slightest stimulus, and are orderly and coördinated.

Limuli that have had part or all of the brain extirpated, no longer seek the mates from which they were taken when operated. In this respect, I found that they differ from those Limuli that had only the olfactory vesicle (sub-frontal sclerite) removed. The latter are still able to find their mates. Proving that the olfactory vesicle does not aid the male to find the female, as it was thought it did (XX).

(d) If we cut between the ganglia of the collar, the animal moves toward the injured side, but only until the wound is healed. No other change in any of the functions seems to have been produced by the operation.

If, however, one or more ganglia of the collar (*C* Fig. 1) are extirpated, the appendages corresponding to the ganglia become paralyzed. Showing that each ganglion controls the activity of the corresponding appendage.

(e) A *Limulus* that has the post-oesophageal ganglion injured, moves in an awkward manner. The nerves that go to the last thoracic appendage, and to the muscles that extend abdominal carapace in walking, are injured in the operation. Hence it is unable to extend the abdominal part. It is very inactive, lying for days in the same spot. Otherwise it does not differ from a normal animal.

(f) In *Limulus* that had the entire brain, post-oesophageal ganglion, and with the exception of three ganglia, the collar extirpated, it was found that the three appendages corresponding to the three ganglia that were left intact, would make the proper masticatory movements, and that food placed in its mouth was swallowed. The post-mortem, made three weeks after the operation, showed that the muscular part around the mouth had regenerated.

To recapitulate:—

1. Lesion or loss of part or the entire brain, oesophageal collar, or post-oesophageal ganglion, has no influence on the respiratory movements.

2. When the abdominal cord is cut anterior to its first ganglion the plates are no longer affected by external stimuli anterior to the lesion, nor are parts anterior to the injury influenced by stimuli on the gill-plates. The gill-plates all move in the same rate, extent, and rhythm.

3. Severing the cord, for example, between the second and third ganglia, or extirpating a ganglion, so that there is an anterior division connected with the brain and a posterior division of the plates separated from it, the rate of respiratory movements of both divisions under ordinary conditions is practically the same. After the animal is made to exercise, after being fed, operated on, or irritated, the rate of the different divisions is altered, but in like direction. For instance, after exercise both divisions of plates inspire at increased rate. After feeding or being irritated the rate of both divisions increases, but that of the anterior may be more rapid than that of the posterior division. In short, the rate of the different plates is the same when they are stimulated by the condition of the blood alone.

The extent and rhythm of the plates above and below the lesion vary according to circumstances. After feeding or irritation the anterior gill-plates move in greater extent and different rhythm from those posterior to the injury. Cramp movements and interrupted respiration that often occur in the posterior division, rarely appear in the anterior.

Each division has its reflex centres and is not disturbed by cramp or interrupted respiratory movements of the neighboring division.

4. Each ganglion of the cord constitutes an automatic and reflex centre for the respiratory movements.

5. The anterior nerves of the ganglia contain sensory fibres, and the posterior nerves, both sensory and motor fibres.

EXPLANATION OF PLATES I-III.

FIG. 1. This figure represents a ventral view of the principal part of the nervous system of *Limulus*, ensheathed by arteries, and enlarged $\times 2$ from the specimen.

- B.* Anterior oesophageal ganglion or brain.
- C.* Oesophageal collar.
- G.* Post-oesophageal ganglion.
- D.* Abdominal ganglionic cord.
- I-VI.* Ganglia of the cord.
- A.* Anterior ganglionic nerves.
- P.* Posterior ganglionic nerves.
- L.* Cut edge of the gill-plates.
- O.* Cut edge of the operculum.
- a-f.* Integumentary nerves.
- h.* Nerves to the thoracic appendages.
- n.* Nerves to the pencils.
- q.* Nerves to the operculum.
- r.* Nerves to the chilicerae.
- m.* Nerves to the median eye.
- p.* Nerves to the sub-frontal sclerite (olfactory organ).
- l.* Nerves to the lateral eye.
- i.* Nerves to the frontal integument.

FIG. 2. This figure is a ventral view of a *Limulus*, of which the right half of the brain was extirpated, and that had forced movements to the left side. The appendages of the left side are bent on themselves, making the angle between the third and fourth segment smaller than the corresponding angle of the appendages of the right side.

- sf. c.* Sub-frontal sclerite (olfactory organ).
- ch.* Chilicerae.
- ant. vent.* Anterior ventral margin.
- app.* Thoracic appendages.
- ceph. car.* Cephalo-thoracic carapace.
- pen.* Pencils.
- o.* Operculum.
- cox.* Coxae.
- 1-5.* Gill-plates.
- ab. car.* Abdominal carapace.
- p. a. c.* Posterior abdominal corners.
- s.* Spine.

FIG. 3. This figure is a ventral review of the gill-plates in cramp position.

- o.* Operculum.
- 1-5.* The gill-plates.

FIG. 4. This figure represents the dorsal view of a gill-plate — natural size.

- g.* Gill-books.
- s.* Parabranchial stigmata.

A MICROSCOPICAL STUDY OF THE NERVE CELL DURING ELECTRICAL STIMULATION.

C. F. HODGE, PH.D.

Experiments on spinal and sympathetic ganglion cells of the frog.

THE purpose of the present series of experiments is to observe continuously the process of fatigue in the nerve cell. Up to the present series my method has consisted in studying the cells at the end of a period of fatigue and only after the tissue had passed through the usual processes of preparation for the microscope. From the first, it has been my desire to study the process continuously in the living cell. A report of former work upon the subject of changes in ganglion cells, due to electrical stimulation and daily fatigue, may be found in a previous number of this Journal.¹

METHOD.

At first an attempt was made to observe the cells *in situ* with circulation disturbed as little as possible. This was done by removing the viscera from in front and a portion of the spinal column, muscles, etc., from the back, tying all blood vessels, thus leaving the pleuroperitoneum with its blood vessels and the sympathetic chain intact. Small frogs were used, but the large pigment cells in the pleuroperitoneum itself, and especially along the blood vessels and sympathetic cord, made it impossible to get a clear view of the sympathetic ganglion cells. I was obliged to abandon the method.

It thus became necessary to remove the ganglia from the body and free them from their pigmented capsules. In consequence of this the following method was adopted, and it has proved more serviceable than was anticipated. The special

¹ Hodge. A Microscopical Study of Changes Due to Functional Activity in Nerve Cells. *Journal of Morphology*, Vol. 7, p. 95. Boston, 1892.

apparatus employed can best be understood in connection with a typical experiment.

A pair of spinal or sympathetic ganglia is quickly excised from a freshly pithed frog. Each of these is placed immediately in a drop of normal solution on a "stage-electrode" of the following construction (Fig. 1): A plate of thin, clear glass (*A*) of shape indicated in figure and of a size to be conveniently clipped to stage of a Zeiss microscope stand, larger size, has two grooves cut in its under surface from margin to near centre. These are made deep enough to conceal two fine

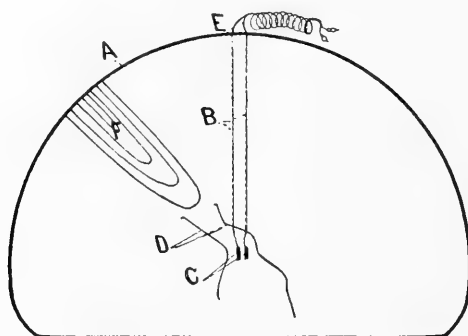


FIG. 1.—MICROSCOPE STAGE ELECTRODE.

- A.* Glass plate.
- B.* Two platinum wires laid in grooves on under side of plate and rising through plate to be exposed on upper surface to serve as stimulating tips at (*C*).
- C.* Exposed portion of electrodes.
- D.* Platinum wires to support cover-slip.
- E.* Wires to induction coil.
- F.* Trough ground in glass plate to carry off stream of normal solution.

platinum wires, which are cemented in them (*B*). A half cm. beyond central end of grooves the platinum wires are brought to upper surface of glass through two needle holes about two mm. apart. The ends of the wires are then bent down into needle-hole pits in the upper surface of the plate about two mm. beyond the first perforations. Shallow grooves in the upper surface are made to connect the holes of each pair, so that the platinum wires come to lie half exposed on the upper surface of plate for a distance of two millimeters (*C*). They are then filed off level with surface of glass. This portion of

Fig. 1.

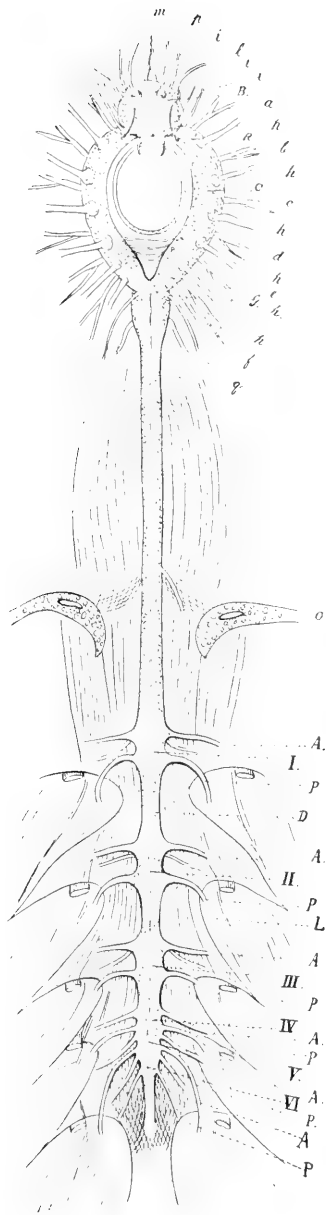


Fig. 2.

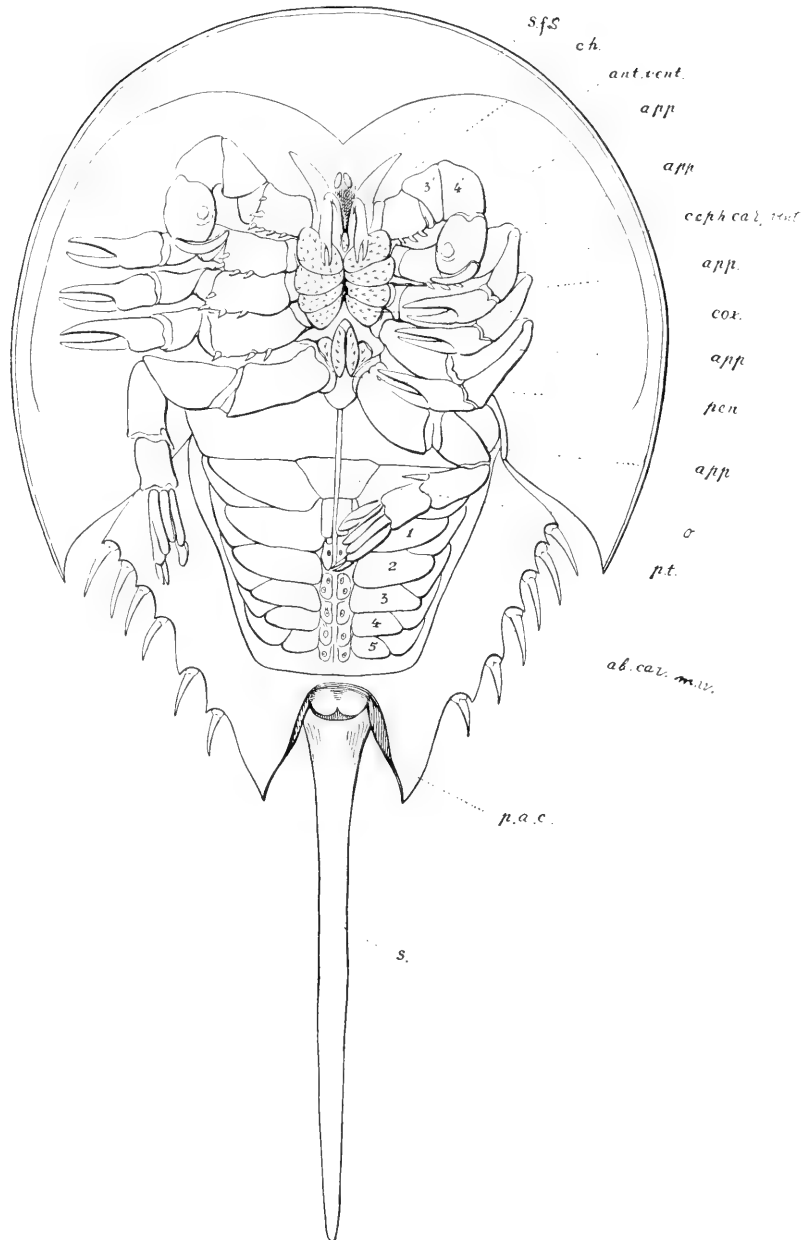




Fig. 4. *s.*

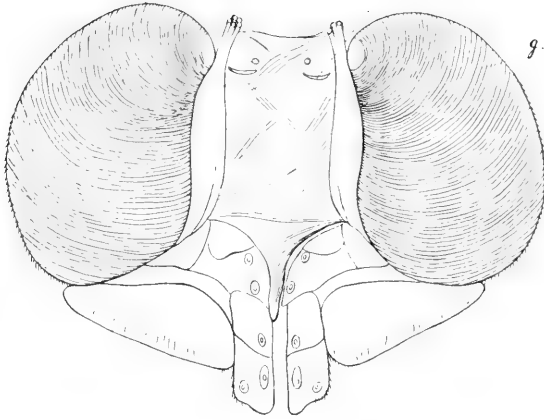
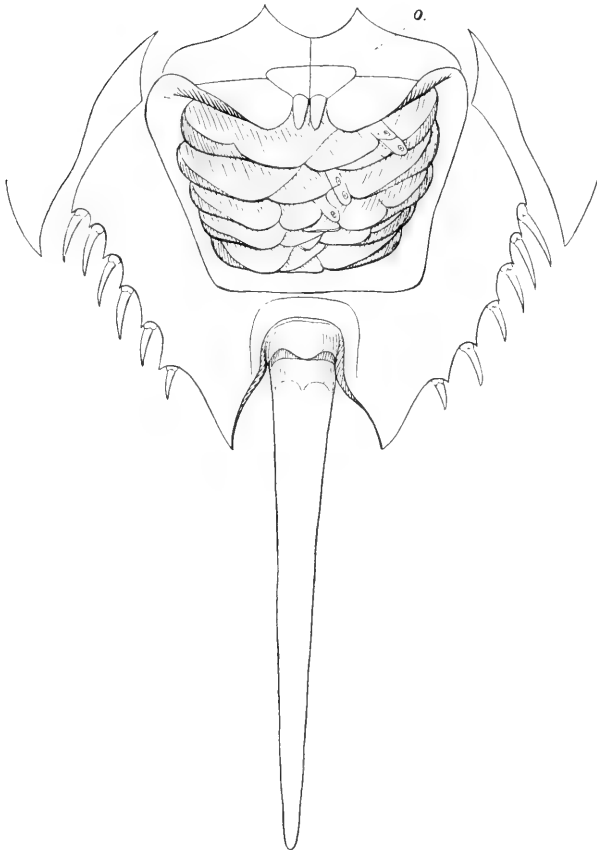


Fig. 3.





the wires, of course, acts as the electrodes, and the preparation is arranged so that either the ganglion lies between the electrodes, or its nerve lies over them. The other end of each platinum wire is soldered to an insulated copper wire which goes to secondary coil. Two other platinum wires (*D*) support the cover-slip. These, for convenience, are hooked into pits in the glass, and are so bent and adjusted as to direct the stream of normal solution over the specimen.

With the plan of this simple electrode understood, the remainder of the method may be seen at a glance in Fig. 2.

Each electrode is clipped to its microscope and a stream of normal solution is drawn from flask (1) by means of glass syphons (2,2') which end in finely drawn nozzles. These are placed at edge of cover-slip opposite trough (*F*), Fig. 1. The stream from (*F*) drips into beaker (3,3'). Other parts of figure need no explanation.

We now have, living in the same fluid, corresponding ganglia of the same animal. A group of cells, as nearly alike as possible, is sought out in each preparation and brought to centre of field. A cell, or several cells in each, are measured and outlined with aid of camera, and then electrical stimulation is applied to one of the preparations, and not to the other. Both are watched, and at intervals measured and drawn. Two cameras, each attached to its microscope, were used at first (as shown in Fig. 2). To render the two pictures more comparable, avoiding thus any difference in the angle of the mirrors, a camera was clamped to the eye-piece, with mirror firmly set, and eye-piece and camera were lifted together from one microscope to the other.

Stimulation was interrupted, 15 seconds' work alternating with 45 seconds' rest, the primary circuit current being made and broken by a "Lombard clock interrupter." Four Daniel cells, supplying current of .2 ampere were used throughout. The induction coil used was one of Kruger's (10305 U.). Aside from the electrode and device for supply of normal solution the apparatus was made as similar as possible to that used in my earlier experiments and fully described in the paper just cited.

At end of desired time both control and stimulated ganglia were placed together in osmic acid and teased in glycerine for further study and preservation.

In all, 33 experiments have been made. These have varied in duration from 15 minutes to 6 days. As formerly reported,¹ they were divided into a winter series, made between Nov. 25, 1892, and Dec. 24, and a summer series, made from June 14

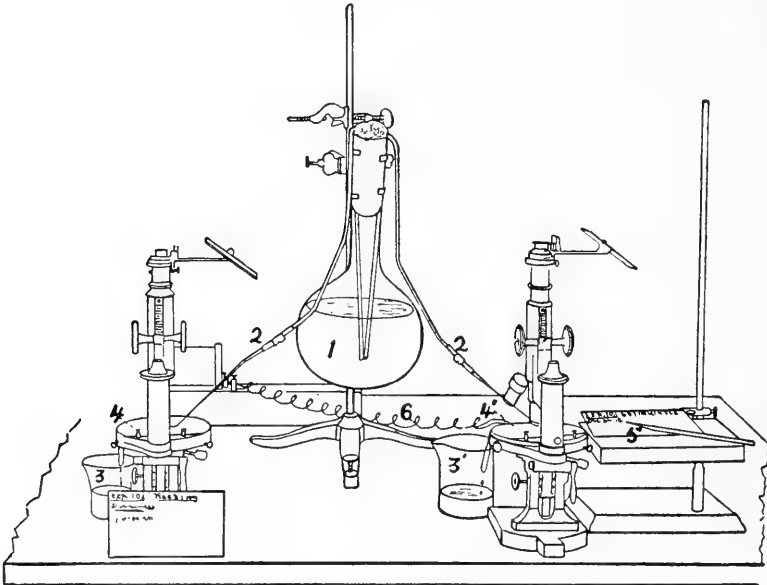


FIG. 2.—SETTING OF APPARATUS FOR GANGLION CELL STIMULATION.

- 1, Flask of normal solution.
- 2, 2' Glass syphons which lead solution to edge of coverslip.
- 3, 3' Beaker to catch drip.
- 4, 4' Stage electrodes in position.
- 5, Drawing stand to hold card at level of stage.
- 6, Wires from right-hand microscope to secondary coil.

to Sept. 3, 1893. Since no marked difference in results has borne out this division, I have substituted for it a grouping of the experiments according to their purpose and nature. It is intended to give in the following table a condensed statement of the whole research. Each experiment bears its date, but for convenience of reference the numbers have been given to the experiments as they stand in the table. The time of day

¹ Pan-American Congress, Physiological Section, Sept., 1893.

is written as an exponent of the date, and since all experiments were made during daytime, no designation of A.M. or P.M. is required. As in previous work, interest centres chiefly about the nucleus. Change in this is expressed in percentage of loss or gain, using the original volume as 100 per cent. Time in hours and minutes, from beginning of experiment, is written as an exponent of the shrinkage per cent. Where several cells were watched, each bears its proper number throughout the experiment. Controls enter the table only in case some change is noted in them. No significant change in size of cell body was observed, hence their omission from table. Experiments 1 and 2 were made with cells of sympathetic ganglia. In all the rest the spinal ganglia were used.

[illegible]

NO. OF
EXP.

DATE.

10.55
13 Jan. 19

Frog similar to 12 — no nuclei or nucleoli visible in any of the cells, although they appear clear and free from pigment. Stimulation applied, and in 20 minutes faint outline of nuclei became visible. After 4 hours and 30 minutes' stimulation faint irregular outline of nuclei visible. — No nucleoli to be seen.

		POSITION OF COIL.	NORMAL SOLUTION USED.	SHRINKAGE IN VOLUME OF NUCLEUS.						
14	2.15 Aug. 14	10 cm.	NaCl 0.65 %	0.30 46 %						
15	5.00 Aug. 14	13 cm.	"	0.30 24 %		1.00 42 %				
16	11.05 Aug. 29	10 cm.	Ringer's solution	1.20 48 %						
17	10.25 Aug. 30	10 cm.	"	0.10 35 %	0.20 58 %	0.30 61 %	0.50 65 %	1.20 69 %	2.45 74 %	
18	9.00 Aug. 30	13 cm.	"	0.23 23 %						
19	2.22 Aug. 30	13 cm.	"	0.10 26 %	0.30 42 %	0.40 51 %	2.45 65 %			
20	9.33 Aug. 31	13 cm.	"	0.12 25 %	0.35 45 %	0.50 54 %	1.50 54 %			
21	11.10 Sept. 2	13 cm.	"	0.15 22 %	0.30 37 %					
22	11.45 Sept. 2	13 cm.	"			1.30 48 %				
23	10.29 Sept. 3	12 cm.	"	0.30 18 %	1.00 29 %	1.30 43 %	2.00 43 %	4.44 69 %	6.49 75 %	

Experiments with distal stimulation.

24	3.30 Aug. 5	13 cm.	Salt solution 0.65 % + Calcium phosphate to saturation.	0.15 17 %	Continuous stimulation.					
25	5.00 Aug. 13	10 cm.	Salt solution 0.65 % + Calcium phosphate to saturation.	0.15 33 %	Same cell as 24.					

Experiments with central stimulation.

26	4.40 Aug. 13	10 cm.	Salt solution 0.65 % + Calcium phosphate to saturation.	0.15 12 %	Continuous stimulation.					
27	4.45 Aug. 5	10 cm.	"	0.15 0 %	Continuous stimulation.					
28	Aug. 13	10 cm.	Salt solution.	0.15 30 %	Continuous stimulation. Ganglion almost touching elec- trode					
29	9.45 Sept. 2	13 cm.	Ringer's solution.	0.15 0 %	Continuous stimulation.					
30	10.30 Sept. 2	13 cm.	"	0.15 0 %	Continuous stimulation.					

Experiments with potassium tartrate solution.

31	10.50 Aug. 28	10 cm.	Salt solution 0.65 % saturated with calcium phos- phate + 0.1 % potassium tartrate.	0.22 14 %						
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Nucleolus moved about in nucleus with continuous change of shape, and finally fragmented and disappeared.

Nucleolus of control was seen at first to change from crescent-shaped to round, in which condition it remained.

NO. OF EXP.	DATE.	POSITION OF COIL.	NORMAL SOLUTION USED.	SHRINKAGE IN VOLUME OF NUCLEUS.
32	1.36 Aug. 28	10 cm.	Same solution.	0.30 39%
				Behavior of nucleolus similar to 31.
33	2.45 Aug. 28	10 cm.	Same solution.	0.30 30%
				Behavior of nucleolus same as in 31 and 32.

The most striking feature of the table is perhaps its great irregularity, the lack of any apparent relation between amount, duration, or intensity of stimulation and effect in change of nucleus. This is in apparent disagreement with results of former work, in which a quantitative relation between stimulus and shrinkage of nucleus is evident. It is, however, easy to show that the lack of agreement is only apparent. In the former experiments we were dealing with definite amounts of stimulus given in the same way to corresponding nerves which, in turn, led the stimulus to corresponding ganglion cells. The point to be emphasized is that the cells were in position in the body under practically the same conditions of blood supply, and in similar relation to the stimulus. In the present series, the cells are removed from the body, placed in a stream of solution which it is impossible to make equal to the different cells, and in an electric field the intensity of which in any point it is impossible to regulate or even estimate. Taking these important variables into account, the evidence for quantitative relations between stimulus and effect is possibly as strong as we should expect to find it. The measurements from which the percentages in the table were computed were made, long and short diameters of nucleus as usual, with no thought beyond accuracy of each measurement.

Differences and similarities between different experiments may be seen more clearly by plotting the percentages in the table in the form of curves. This was done for all the experiments. Four of the most comparable of the curves are reproduced in Fig. 3, drawn from experiments 18-21. Position of coil and solution are the same in these experiments, yet the course run by each cell in its fatigue is distinct, though similar in character. By far the majority of the curves are of the

same general form. They show that the cell tires rapidly at first, then more gradually, to a condition of fatigue, complete, at least, so far as our present method is able to indicate. This is also in apparent variance with curves derived from a former series of experiments, in which fatigue was rapid at first, then gradual, then more rapid again. A slight tendency to this form of curve is found in experiments 6, 8, 9, 18, 23, these

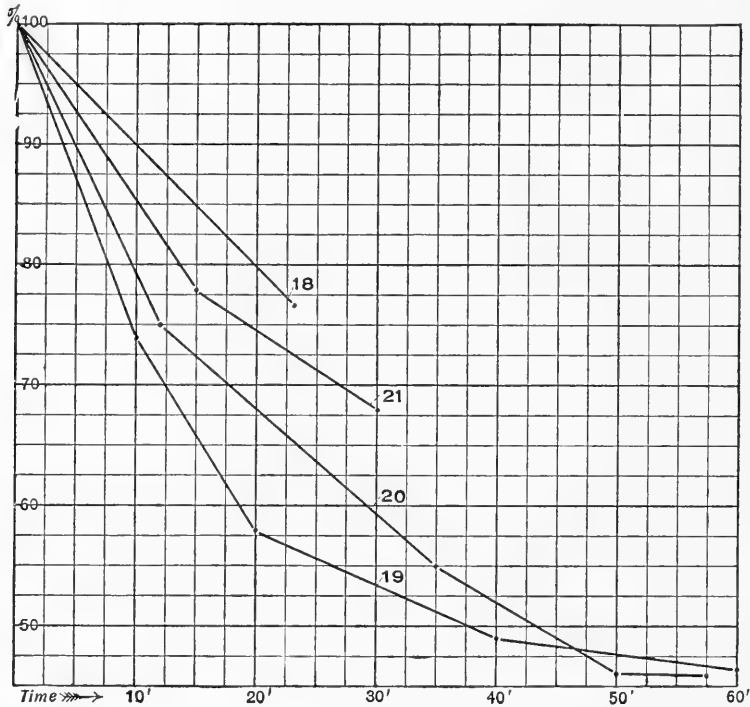


FIG. 3.

all showing slight secondary acceleration. Considering the circumstances of the two series of experiments, no correspondence can be expected. In the present series, the cells are not living in a nutrient solution, but in a normal non-nutrient solution which is able, in all probability, to carry off by diffusion waste matters of the cell's metabolism. Consequently the nucleus shrinks more rapidly, and also more continuously, to a condition of complete fatigue. As more fully discussed in a previous paper, there are reasons for believing that cells in

presence of nutriment carry on the processes of anabolism and katabolism at the same time. Under the conditions of our present experiments, anabolism is impossible, while katabolism is possibly unimpeded or accelerated. Note the slight shrinkage which occurs in a few of the control ganglia.

Shrinkage in nuclei of control cells probably occurred to a slight degree in all experiments where simple sodium chloride solution was used. It never reached the point at which the nucleus began to show indentations. In Ringer's solution, or in salt solution saturated with calcium phosphate, no change in this respect could be detected, even for long periods. This agrees in the main with recent work by Locke¹ upon the influence of so-called "normal" solutions on the form of curve of muscle contraction, salt solution making the curve higher than normal. A slight effect tending toward that produced by stimulation is no more than we should expect in transferring ganglion cells from blood to any normal solution. In experiment 9 the control nucleus takes a peculiar course. It is seen to swell slightly for the first two hours, it then comes back to normal and continues to shrink, until in seven hours it has lost 19% of its original bulk. I have, as yet, no adequate explanation for this case. It stands alone so far as my observations go. Upon diluting a normal solution with distilled water, I have observed similar swelling of nuclei. This fact, however, throws little light on the present instance.

Experiments 1 to 23 were all made with the single purpose of observing changes which might occur in ganglion cells during stimulation. But few in this group require fuller statement than is already given in the table.

In experiment 3 the cells were kept under observation at intervals, for six days. Camera drawings were made, at least one a day, during the whole time. Measurements were taken to begin with, and at the end of three hours. As the mirror of camera was moved, it is not possible to compute volumes accurately beyond this time. Measurements were omitted under the impression that camera drawings would serve instead.

¹ Locke, *Die Wirkung der physiologischen Kochsalzlösung auf quergestreifte Muskeln*. Pflüger's Archiv, Bd. 54, S. 501-524. Bonn, 1893.

This necessitates a brief description by way of supplementing the table.

The experiment was begun at noon, Dec. 7, and during the first five hours the nuclei decreased in size and became irregular in outline. By the next morning the nuclei had somewhat regained their rounded outline, though remaining small; and appearance of both protoplasm and nuclei showed little or no change for two days, beyond the retraction of the protoplasm from capsule to envelop closely the nuclei. During the fourth day granules in protoplasm and nuclei appeared in violent commotion. My notes for this day contain the words, "cells alive with bacteria." An incrustation of salt had formed around edges of cover-slip in places. In washing this off with distilled water, a little ran under the cover-slip, and the nuclei were observed to swell. It was expected that the "bacteria" would leave little of the cells to be seen next day. On the contrary, by the morning of the fifth day all movement of granules had subsided, and nuclei and protoplasm had regained, to all appearances, their former condition. Upon the morning of the sixth day, however, the nucleoli of two of the cells had entirely disappeared, and the outline of the nuclei in these cells could scarcely be distinguished. In the third cell the nucleus was visible, though faintly, and contained a small speck of still highly refractive nucleolar matter. On tapping the cover-slip lightly to dislodge a mass of *débris* which had floated out so as to obscure some of the cells, they all flew to pieces, and the experiment was at an end.

Experiment 4 also deserves a word of explanation. An attempt was made in this case to obtain, by shoving the secondary coil to 0, the greatest possible effect in the shortest time. Contrary to expectation, however, no change could be observed at first. Very little occurred during the first three hours, and the nuclei remained round and clear during nearly twenty-one hours of this extreme stimulation. Three more experiments, not given place in the table, were tried with coil in same position, and with the same result. Motile protozoa were next submitted to the same stimulation. *Paramoecia* were found to be killed almost instantly. At least they showed

no signs of movement after a few (less than fifteen) seconds' exposure to the current, and speedily disintegrated. Large vorticellae were able to endure more; but after two or three exposures of fifteen seconds each, they invariably succumbed. A number of similar tests were made with gradually decreasing strengths of current, and it was found that with the secondary coil at ten, paramoecia, although considerably startled, did not appear to be injured. Frog's leucocytes were used in a similar way. No relation necessarily exists between strength of current injurious to paramoecia and to frog's spinal ganglion cells; however, 10 cm. was decided upon as the limit of stimulation for remaining experiments.

Experiments 12 and 13 are anomalous. In perfectly clear, slightly pigmented ganglion cells no nucleus or nucleolus was to be seen. This is not peculiar to the June experiments: for even during the winter several cases of the kind occurred. They were, at the time, discarded with the note, "cells not clear, no nuclei visible." The two instances, 12 and 13, are too pronounced to leave unrecorded. They force upon one comparison with the apparently similar disappearance of the germinal vesicle in the ovum at maturation. The fact that on stimulation traces of nuclei, but none of nucleoli became visible is also of significance. But whatever this may be is at present a matter for experiment rather than discussion. The subsequent experiments, 14 to 33, made upon young frogs, *R. temporaria*, which had just passed the tadpole stage, left nothing to be desired by way of clearness of nuclei.

A fair picture of an experiment can hardly be given in a table or by curves. It requires a

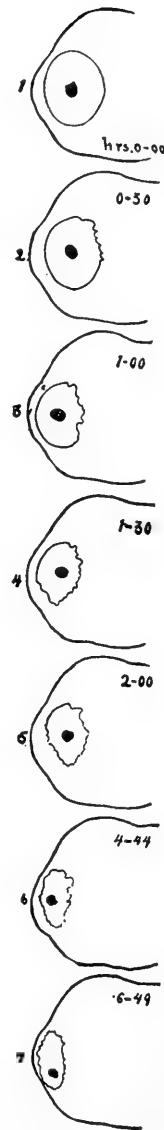


FIG. 4.

Camera outlines of cell, nucleus and nucleolus in Experiment 23. (Zeiss, Oc. 6, obj. 40x 375.)

Time is given in hours and minutes.

series of plates drawn from the living specimen. This has not been done, because I do not feel that my study of cell granulation and its changes during fatigue is at all complete. Hence for the present the accompanying outline drawings, reproduced from camera outlines made during the experiment, may suffice. Fig. 4 represents experiment 23, 1 to 7 being successive drawings of the stimulated cell.

To begin with, we have a typical spinal ganglion cell with nucleus situated eccentrically. The nucleus is clear, slightly oval in shape, but with perfectly even contour, as seen in optical section. After thirty minutes stimulation, 2, Fig. 4, the side toward centre of cell has changed, the outline here having become faint and indented. In 4 the other side has begun to cave in, and so to 7, where we see a shrunken jagged nucleus one quarter its original size. It has come to lie a little closer to the cell capsule, and the cell has not decreased in size to a measureable amount. To enter here more into detail would be to repeat a former description. The experiments are in fact repetition of former work under conditions varied so that we see continuously and in a single cell what we were able to find in a series of different animals and months of experimentation before. Placed in osmic acid the nuclei retain this appearance, which fact completes connection with former work done by the osmic acid method. It should be added, however, that the characteristic darkening of the nucleus as it shrinks does not occur in these experiments.

Considerable attention was directed toward studying changes in the granulation of protoplasm during stimulation. Some of the cells became lighter and clearer. In others this was not so manifest. In experiment 3 the cell under observation contained several prominent oil droplets. During the first two hours one of these was observed to become smaller and smaller, and at last it disappeared. Two more, observed at first to be separate, were seen to have moved together and to coalesce. Teased in glycerine, after treatment with osmic acid, the cell-protoplasm is seen to be pervaded by large irregular light spaces, probably the vacuoles observed in sections. The entire cell is, however, too thick for such study. It

was impossible also to detect indications of vacuolation in the living cell.

During the whole time nuclei were also subject to closest scrutiny in the hope of seeing possibly the movements described by Svierczewski,¹ and of gaining some light as to the function of this organ outside of strictly reproductive processes. While in normal salt solution or Ringer's solution, no movement of any sort, or only slight changes of shape and position, such as are indicated in Fig. 4, could be observed, together with a gradual decrease in size. Connected with this latter it was possible to make out granules in the nucleolus which moved slowly about and in several instances were seen to be extruded into the nucleus. Confirmation of the above was immediately sought in sections of spinal ganglia already in my possession, and was found in abundance in osmic acid specimens stained in safranin. The granules here were stained brighter red than the body of the nucleolus and several were found partially extruded.

In this connection I may briefly refer to experiments 31 to 33, although they belong properly to a subsequent paper. Potassium tartrate, 0.1%, was added to sodium chloride and calcium phosphate solution with a view of giving the cells a trace of potassium and more oxygen, in case there was not enough accessible in the plain liquid. The somewhat unexpected result may be seen outlined in Fig. 5. The different stages represented in 1-4 were all actually observed and camera outlines made in twenty-two minutes. The movements of the nucleolus in this case had every appearance of being amoeboid. The nucleolus of the control cell changed shape somewhat but retained its size, whereas the fragments of the stimulated nucleolus had all dissolved in thirty minutes. Experiments 32 and 33

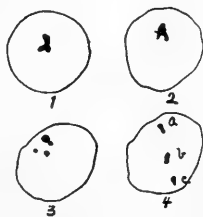


FIG. 5.

Camera outlines of changes in nucleolus in Experiment 31 during 22 minutes stimulation in 0.1 per cent potassium tartrate added to normal solution. *a-c* occurred in 4 minutes. (Zeiss, Oc. 6, obj. 40×375.)

¹ Svierczewski, *Zur Physiologie des Kerns und Kernkörperchens der Nervenzellen des Sympatheticus*. Centralblatt f. d. Med. Wissenschaft., Berlin, 1869. p. 641.

gave exactly similar results, except that the control nuclei were not observed to move.

A number of the briefer experiments were made with the special purpose of testing upon frogs certain results which Vas¹ has recently reported from experiments upon the sympathetic ganglion cells of the rabbit. Among these results Vas describes a swelling of the cell and especially of the nucleus upon continuous stimulation for fifteen minutes, the electrodes being applied to the cervical sympathetic 3 cm. below the superior cervical ganglion. The nuclei of these cells became swollen and clear; exactly the reverse of my results for spinal ganglion cells after a longer period of intermittent stimulation. It is of interest to note that this diametrically opposed result is obtained by stimulating the nerve in the reverse direction to that of the normal passage of the nervous impulse. Hence in these experiments stimulation was applied to the dorsal root between cord and ganglion.

Of the seven experiments under this head, four showed no change in the appearance of nucleus, two, slight change, 7-12%, one, marked change of about 30%. All change observed was toward shrinkage, with no tendency to swell. It should be added, however, that the present method affords no adequate test of Vas's results, since (if stimulation the reverse way tends to produce swelling, and if this be anabolic in character) no anabolism would be possible in a non-nutrient solution. In the two experiments with slight change the ganglia were near the electrodes, in the one with marked effect it almost touched one electrode, so that the change produced was in all probability due to irradiation of stimulus.

In the present series no attempt has been made to do more than to test under new conditions the validity of previous work. A method has been devised by which a much easier and more complete and satisfactory demonstration of fatigue changes in ganglion cells may be made. Former work has found confirmation with the additional result that the effect produced by hours' stimulation of a nerve in the body may be obtained in a

¹ Vas, Ferd, Studien über den Bau des Chromatins in der sympathetischen Ganglienzelle. *Archiv f. Mik. Anat.* Bd. 40, S. 375. 1892.

few minutes by removing the cells to a non-nutrient solution and placing them between the electrodes.

And concluding, I may call attention to the fact that with the above method we have a means of studying the action of any chemical substance, medical or nutrient, upon the nerve cell itself. With modifications for temperature, it may serve equally well for mammalian tissue.

PRELIMINARY ACCOUNT OF THE CELL LINEAGE OF AMPHITRITE AND OTHER ANNELIDS.

A. D. MEAD.

Cleavage.—The egg of *Lepidonotus* is small and quite free from yolk, develops into a very active trochophore, and, as one might expect, the cleavage is extremely regular. The first furrow cuts in at the animal and vegetative poles at the same time. At the four-cell stage all the cells are of the same size, and in no way distinguishable one from the other. In the later stages (up to seventy cells or more) the quadrants which these cells represent also appear to be exactly similar, so that whatever phenomena take place in one quadrant take place, at the same time, in each of the others. The number of cells increases, in geometrical progression up to sixty-four, by the practically synchronous division of all the cells at each successive stage. The result is the attainment of two, four, eight, sixteen, thirty-two, and sixty-four-cell stages. Up to the sixty-four-cell stage every cleavage furrow cuts the meridian obliquely (spiral cleavage of Lang, Wilson, Kofoed, *etc.*), while the direction of the cleavage reverses with each successive division.

With the attainment of the sixty-four-cell stage this regularity suddenly ceases. Some of the cells now acquire cilia and stop dividing forever (prototrochal cells); others do not divide for a long time; in others, which form the cross as in *Nereis*, the next cleavage plane coincides with the meridian; still others, including the rosette cells, continue to divide, the direction alternating as before.

An invaginating plate of eight cells is formed at the vegetative pole. The cells are the four "macromeres," and the four "micromeres of fourth generation."

At the thirty-two-cell stage the polar bodies usually pass into the apical cells, where they can be followed for a long time. They eventually disappear within the cells.

The eggs of *Amphitrite ornata* are much larger than those of *Lepidonotus*, and from the first there is a segregation of yolk at the lower pole. The direction of every cell-division up to the sixty-four-cell stage is the same as in *Lepidonotus*. There is also almost as great regularity in the time of the divisions.

There is not, however, the same regularity in the size of the cells. The first division is unequal, and the divisions up to eight cells are very similar to those of *Nereis*. The descendants of the large macromere *D* of the eight-cell stage (I shall use Wilson's nomenclature in this paper) are different, both in absolute and relative size, from the corresponding cells of the other quadrants, and exhibit an increasing tendency to more frequent division.

At the sixty-four-cell stage the regularity in the cleavage stops. Sixteen of the cells (prototrochal) at once acquire cilia and never divide again. The four cells which form the cross do so by dividing bilaterally, as in *Lepidonotus*. Other cells divide in a plane which is oblique to the meridian, but in the *same direction* as the previous division, while still others continue alternating in direction of cleavage, as in the earlier stages.

The eggs of the Maldanid *Clymenella torquata* have two or three times the diameter of the *Lepidonotus* eggs, have a large amount of opaque yellow yolk, and develop into a comparatively sluggish trochophore. The cleavage is extremely regular up to the sixty-four-cell stage, the direction of each cleavage being the same as in *Lepidonotus* and *Amphitrite*. In the relative size of the cells, and in the time-rythm, it is very similar to *Amphitrite*. The cross and rosette are formed in exactly the same manner. Again, as in *Amphitrite*, immediately after the sixty-four-cell stage certain cells divide obliquely to the meridian, but in the same direction as the previous cleavage.

While in *Lepidonotus* the cells of the eight-cell stage are all of nearly equal size and clearness, in *Scolecopsis viridis* the four apical cells are relatively very small and perfectly transparent; the four vegetative cells, on the other hand, are huge in proportion, and opaque.

Nevertheless, in *Scolecoplepis* the eight cells divide synchronously and in the same direction as in *Lepidonotus*. Twelve of the sixteen resulting cells also divide together, and in the same direction as in *Lepidonotus* and *Amphitrite*. I have not observed the division of the other four. Since *Scolecoplepis* has a suppressed trochophore, it is interesting that these and other cells, which in other forms give rise to the prototroch, are minute and slow to divide. The apical rosette is formed in the typical fashion.

Germ-Layers.—In *Amphitrite*, at the sixty-four-cell stage, the germ-layers are segregated into definite cells. d^4 ($=M$) forms the mesoderm, a^4 , b^4 , c^4 , and A , B , C , D , form the entoderm, the latter cells each dividing once before the invagination. The rest of the cells form ectoderm.

The mesoderm cells M and M , products of d^4 , sink into the segmentation cavity and produce a pair of typical germ-bands by teloblastic budding. They do not bud off small cells upon the surface before they sink in, as has been found in *Nereis*, *Unio*, *Umbrella*, and other annelids and molluscs. However, when they have become elongated, and their nuclei have moved inward to about the middle of the cylindrical cell-body, each buds forth a small cell which is carried into the segmentation cavity with the large one and remains at the anterior end of the germ-band. *The axes of the spindles in these divisions lie in the shortest diameter of the cells and apparently in the direction of greatest pressure.*

Larval Organs.—In *Amphitrite*, the *prototroch* consists at first of sixteen cells, which are differentiated and become functional in the sixty-four-cell stage. They lie in four separate groups, of four cells each, and are descended from the "first generation of micromeres." In *Nereis*, according to Wilson, only twelve of these sixteen cells are involved in the formation of the prototroch. Later the prototroch is completed by the addition of nine more cells from the "second generation of micromeres," in the quadrants A , B , and C , respectively. No further cells are added in the D quadrant, so an interruption still persists in this region—"dorsal interruption."

The history of the prototroch in *Clymenella* is similar in every detail to that of *Amphitrite*.

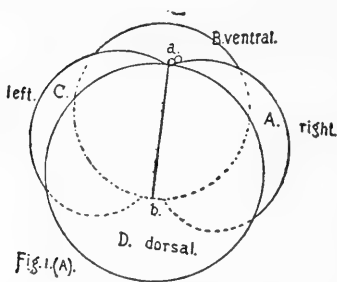
The *paratroch* in *Amphitrite* consists of four cells, one of which is the descendant of x^1 , the other three of x^2 . They are differentiated some time after the completion of the prototroch. The cells at first lie in the arc of a circle at the posterior lip of the blastopore, two on either side of the middle line (Fig. II). Two of them may be called dorsal, and two ventral, paratrochal cells. The dorsal ones unite in the median line. The ventral ones, on the contrary, are widely separated at first, and are later brought together by the general lateral movement and concrescence to be described below.

Within the arc are small cells which later give rise to the proctodaeum—*terminal cells*. The paratroch persists until the larva has attained five or six setigerous segments.

Mucous Glands.—The apical cross in *Amphitrite* is so exactly similar to that of *Nereis* that no description is necessary. The cells, $c^{1.5}$ and $d^{1.5}$, which in *Nereis* become the "head kidneys," in *Amphitrite* give rise to a pair of huge mucous glands. Other mucous glands occur.

AXIAL RELATIONS.

In *Amphitrite*, in the four-celled stage, a vertical plane passed through the middle of the cells *B* and *D*, corresponds to the sagittal plane of the future animal (Fig. I).



The apical pole, indicated by the position of the polar bodies, corresponds to the future anterior end, the future posterior end being near the opposite (vegetative) pole. The cells *A* and *C* represent, then, the right and left quadrants of the

early trochophore; *B* the ventral, and *D* the dorsal quadrants, respectively. But in the elongated trochophore the descendants of the cell *D* come to occupy not only the whole dorsal region of the body, but at the posterior end, the lateral and ventral regions as well.

At the sixty-four-cell stage we can first speak of the *trochophore*.

At this time the germ-layers are segregated into definite cells, and the primary prototroch is functional, so that the animal swims about.

The trochophore is now spheroidal. The rosette cells, with polar globules still attached, mark the anterior end, while the posterior end lies nearly 180° away at the *posterior end* of the *somatic plate*, i.e., the cells of the d^2 or X group (ventral plate, Wilson).

The primary prototroch, and the cells which complete the prototroch, constitute a transverse band about the trochophore, separating the anterior from the posterior hemispheres, except at the interruption in the mid-dorsal region.

The form of the anterior hemisphere and the relative positions of its component areas, remains comparatively constant throughout the history of the trochophore; as does the prototroch itself. This offers a very satisfactory means of orientation with reference to the metamorphosis which takes place in the posterior hemisphere.

Behind the prototroch occur the most significant changes, affecting the position of cells and areas, and also the form of the posterior hemisphere; these changes being coincident with the formation and development of the *trunk*.

In the sixty-four-cell stage the ectoderm of the trunk, dorsal, lateral and ventral, is represented by the three cells of the X group. These cells are restricted to the dorsal part of the posterior hemisphere, where they form a thick plate (*somatic plate*). A large part of the surface area lying immediately in front of the plate is occupied by the cells of the mesoderm and entoderm.

There is a small segmentation cavity.

A line, *acb*, (Fig. II) passed through the trochophore from the apical pole, perpendicular to the prototroch, will fall in the area occupied by the mesoderm cell. I will call this line the vertical axis. p represents the morphological posterior end of the body. Thus, the antero-posterior axis, *acp*, does not coincide with the vertical axis, but is slightly bent.

Soon, however, the mesoderm leaves the surface and sinks into the segmentation cavity as described above. As it does so the somatic plate extends backward and meets the entoderm plate. This slight movement carries the morphological pos-

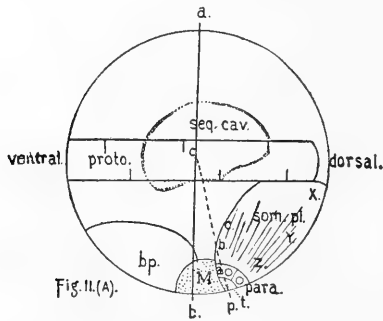


Fig. II.(A).

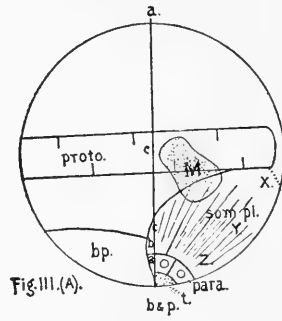


Fig. III.(A).

terior end of the animal to a position 180° from the apical pole (Fig. III). At this point it remains, so that now and henceforth the antero-posterior axis coincides with the vertical axis.

As soon as the mesoderm has sunk into the segmentation cavity the entoderm plate also begins to invaginate. The cells gradually elongate, so that the entoderm plate becomes thickened, while its surface area diminishes. This process continues until at length the entoderm cells withdraw entirely from the surface and lie within the segmentation cavity, and the blastopore has closed.

At the same time with the thickening of the entoderm plate and the diminution of its surface area, there goes the thinning-out of the somatic plate by successive cell divisions and a lateral extension of its surface area. In fact, the lateral edges of the somatic plate follow the retreating boundary line of the blastopore, and, when the blastopore has closed, meet in the mid-ventral line, uniting with each other by a process of typical concrescence, and separate the blastopore from the *terminal cells* (Fig. IV).

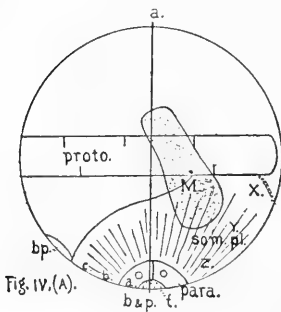


Fig. IV.(A).

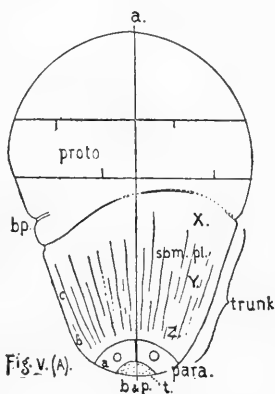
Long before the closure of the blastopore the paratrochal cells are fully differentiated, and enclose the terminal cells,

which later form the proctodaeum; so that there is, from first to last, no uncertainty about the location of the posterior end of the animal.

In order to be sure that the ventral plate is formed by the *lateral extension* and concrescence of the sides of the somatic plate, I followed each cell division on the posterior hemisphere until the blastopore had nearly closed, and some of the somatic-plate cells from either side had actually come together in the mid-ventral line. From this time numerous landmarks make it possible to follow the cells in groups to a much later period.

The foundation of the trunk region is now laid, and the trochophore begins to elongate by a rapid growth at the posterior end, so that the distance between the prototroch and paratroch rapidly increases (Fig. V).

Thus, all the ectoderm of the trunk is formed from the somatoblast d^2 or X Fig. V. (A).



The points to which I have called attention will stand out more clearly if we compare briefly the axial relationships of Amphitrite with those of Nereis, as described by Wilson.

1. In Amphitrite the prospective sagittal plane passes through B and D of the four-cell stage. In Nereis it coincides with the second cleavage furrow (Fig. I).

2. In Amphitrite the cells which form the proctodaeum—terminal cells—are descendants of the somatoblast X . In Nereis they (the pigment cells) “are certainly in part the offspring of the primary mesoblast.”

3. In Amphitrite those descendants of the somatoblast X which from the first lie farthest from the prototroch, maintain this relative position, and form the *posterior* end of the trunk; while those cells which lie nearest the prototroch at first also maintain this relative position, and form the *anterior* part of the trunk. Only a lateral shifting of the regions takes place. There are no “posterior teloblasts” and no cells which

can be homologized with the neuro-nephroblasts of the Oligochaetes and Hirudinea.

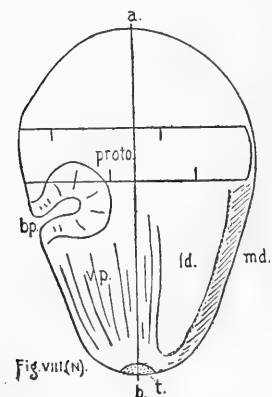
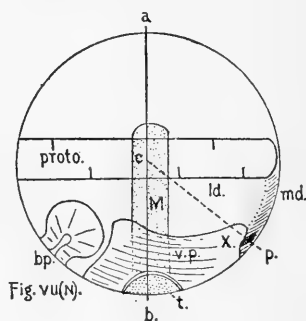
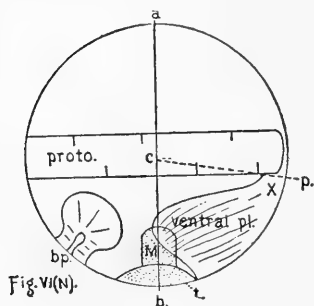
In *Nereis*, on the other hand, the posterior end of the body is represented by the cells of the *X* group,—"posterior teloblasts,"—which at first lie in contact with the prototroch 90° from the pigment area (Fig. VI, *t*). These cells Wilson considered as being without doubt the homologues of the neuro-nephroblasts.

By a shifting of the neural axis through 90° they are carried down to their definitive position near the pigment cells, the space which they vacated being filled by the rapidly-growing latero-dorsal and mid-dorsal regions (*ld* and *md*, Figs VII and VIII).

Thus, from the standpoint of the prospective rôle of their component areas, the early somatic plates of the two forms are inverted with respect to each other, the anterior end of the plate in *Amphitrite* being equivalent to the posterior end in *Nereis*.

4. In *Amphitrite* the somatoblast *X* gives rise to the *whole of the trunk ectoderm*—ventral, lateral, and dorsal; in *Nereis* only to the ventral and mid-dorsal ectoderm, the latero-dorsal regions being formed from $a^{2.1}$ and $c^{2.1}$, respectively (Fig. VIII). In *Amphitrite*, it will be remembered, almost the entire substance of $a^{2.1}$ and $c^{2.1}$ enters into the prototroch.

The dorsal interruption in the prototroch of *Amphitrite* does not persist after the trochophore begins to elongate; but the cells on either side come together in the middle line, and thus the



interruption is obliterated. No new prototrochal cells are formed. While the interruption still persists, some of the cells of the anterior hemisphere migrate through the opening and come to lie immediately behind the prototroch.

MARINE BIOLOGICAL LABORATORY, WOOD'S HOLL, MASS.,
August 10, 1894.

PRELIMINARY NOTE ON THE MATURATION AND FERTILIZATION OF THE EGG OF ALLO- LOBOPHORA FOETIDA.

KATHARINE FOOT.

IN the following preliminary I shall state briefly some of the results obtained from the study of more than two hundred eggs, taken from the cocoons of *Allolobophora foetida*, and supplemented by the study of the earlier stages in the ovary.

The work was begun in the summer of 1893 at the Marine Biological Laboratory, Wood's Holl, Mass.

I express my grateful acknowledgments to the Director, Dr. Whitman, for his valuable assistance, and also to Dr. Conklin, a member of the staff.

The deposition of the cocoons, which I have been so fortunate as to observe several times, will be fully described in a future paper.

The shape of the average egg is shown in Fig. 1. The size at this stage of development varies from .10 mm to .15 mm, and the membrane adheres much closer to the egg than is the case after the polar bodies are formed. When the cocoon is first laid neither polar body has been constricted off; the first maturation spindle is formed, but still occupies the centre of the egg. As it approaches the periphery it occupies positions varying from perfectly radial to entirely tangential, but at the time of division it is either radial or slightly oblique. At each pole of the spindle there is a very pronounced aster.

The spermatozoa are free in the albumen of the cocoon, and they do not penetrate the egg until some minutes (about ten) after the cocoon has been deposited. Fig. 2 shows an average spermatozoon obtained about two hours before the cocoon was laid. The full grown spermatozoon, taken from the freshly deposited cocoon, is about two and one-half times the length of the young sperm represented in the figure; thus the sperm grows with extreme rapidity during this interval of time.

The same method of staining applied to the spermatozoa in these two stages produces very different results. In the first

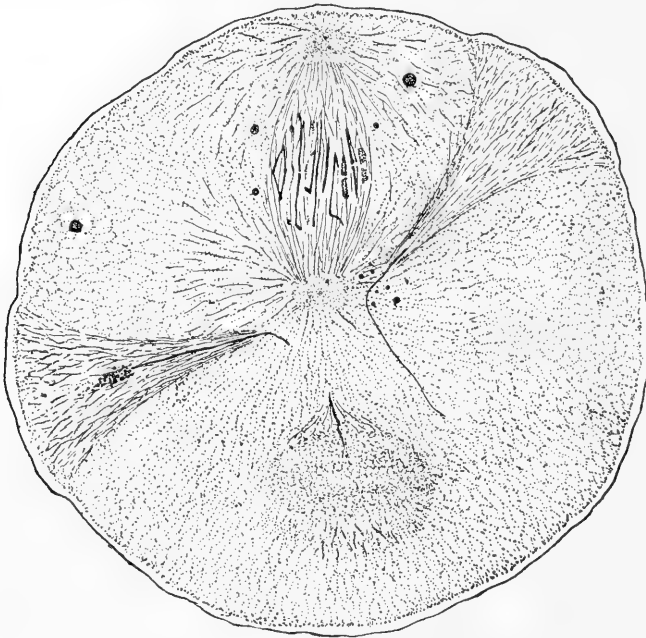


FIG. 1. Optical section of entire egg, showing three spermatozoa entering egg, three cones, nucleoli in the cytoplasm, sperm granules, and first maturation spindle.

stage the tail does not stain; in the second the head and tail stain with equal intensity.

From one to three spermatozoa may enter an egg, each pass-



FIG. 2. Spermatozoon obtained about two hours before deposition of cocoon—showing spine, head, middle piece and tail.

ing through like stages of development until it has attained the pronucleus stage.

The normal time of entrance seems to be after the first maturation spindle has reached the periphery of the egg, but before its division. In some cases, however, they penetrate later, as several specimens show the first polar body already

formed while one or more spermatozoa are penetrating the periphery of the egg.

The area of the cytoplasm through which the spermatozoon enters shows a cone-shaped structure,—Fig. 1. In this case three spermatozoa are entering about the same moment and at various points. The slight projection of the cone beyond the periphery would suggest that it is of the nature of a “cone of attraction”; and in some cases the projection is much more marked than is represented in the figure. At present, however, I do not feel justified in giving a decision on this point, as I have no preparations showing a cytoplasmic extrusion as the sperm comes in contact with the egg, and I have one specimen which suggests that the sperm must enter a definite distance before the cone appears. Fick (5) shows a similar cone-shaped structure in *Axolotl*, which is undoubtedly of the same nature as that of *Allolobophora foetida*, although the former appears later, differs somewhat in relative shape and according to Fick, originates by an invagination. The cone persists until the first polar body is in process of constriction. After that stage of development has been reached the cone has disappeared, and in most cases the sperm aster is seen at the point previously occupied by the apex of the cone. In all cases it is at that point of the spermatozoon immediately posterior to the head,—the point which has been designated as the middle piece.

In a few preparations, where a tardy spermatozoon is relatively near the periphery of the egg, when the first polar body is being constricted off, the aster is forming near the base of the cone while traces of the cone structure still persist. Thus we see that the aster appears immediately on the formation of the first polar body, without regard to the exact location of the middle piece in the egg. In two preparations, where the head of the sperm is penetrating the egg, at the time the first polar body is being constricted off, there is no aster present, and thus we have negative evidence that the middle piece is necessary to the formation of the aster. This seems to be a confirmation of the results obtained by Calkins (4) in his study of the spermatogenesis of *Lumbricus*.

When the spermatozoon first enters the egg, in most cases it takes a direct course towards the central aster of the spindle (Fig. 1). When it has nearly reached the aster it swerves away, as though it were repelled, and continues this curved course until its middle piece has reached the point in the curved path nearest to the aster (Fig. 1). When the sperm has penetrated a definite distance into the egg, on each side of

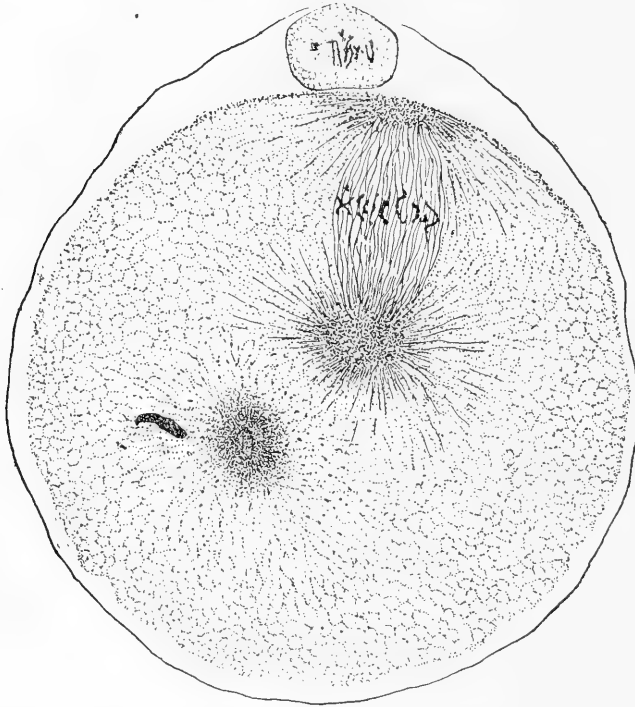


FIG. 3. Optical section of entire egg, showing first polar-body, second maturation spindle, contracted head of sperm and sperm aster.

its thread-like head are distributed dark, round bodies, which stain as intensely as the sperm head itself (Fig. 1). The origin and fate of these bodies I have not yet sufficiently investigated. I shall call them sperm-granules. The early movements of the spermatozoon would suggest that the middle piece of the sperm and the central aster of the egg attract each other; but if this is correct, the peripheral aster of the

spindle differs constitutionally from the central, as it exerts no influence on the sperm. In one preparation a spermatozoon has entered near this aster and is in process of passing it, on its way towards the central aster.

After the complete separation of the first polar body the head of the sperm is found to be contracted into a relatively short, thick rod distinctly separated from its aster (Fig. 3), and occupying a position in relation to it, varying from the radial to tangential. This separation seems to be caused by the thickening and consequent shortening of the head, as in earlier stages the head and aster are almost in contact and the former is relatively longer and more thread-like. During the process of constriction of the first polar body (seen in several specimens), the successive stages of contraction of the head of the sperm can be followed.

The chromosomes of the first maturation spindle are extremely pronounced; their shapes and number can therefore be accurately determined. The individual chromosomes of the spindle shown in Fig. 1 have been selected from three specimens, in order to combine in one spindle some of the various forms seen.

There are eleven chromosomes in the first maturation spindle (Fig. 4), eleven in the first polar body, eleven in the second maturation spindle (Fig. 5), eleven in the second polar body, and eleven in the egg after the second polar body is constricted off.

The second maturation spindle, when it has reached the metaphase, is quite as large as the first spindle; therefore, as Boveri (2) suggests, its achromatic substance

must be replaced from the cytoplasm. The position of the spindle is radial, or slightly oblique, and the two asters are as pronounced as those of the first spindle.

Polar Bodies.—Two are constricted off from the egg and a third is formed by a division of the first. The three have about the same degree of transparency and the same general



FIG. 4. Polar view of first maturation spindle.

FIG. 5. Polar view of second maturation spindle.

aspect. Their shape is either round or slightly flattened (Figs. 3 and 6). The first is about twice the size of the second, and as a rule divides before the second is constricted off, though this is not invariable. When the first divides equally the chromatic substance appears equally distributed between the two halves; but frequently the first polar body breaks up into three or four parts before the second is formed. In these

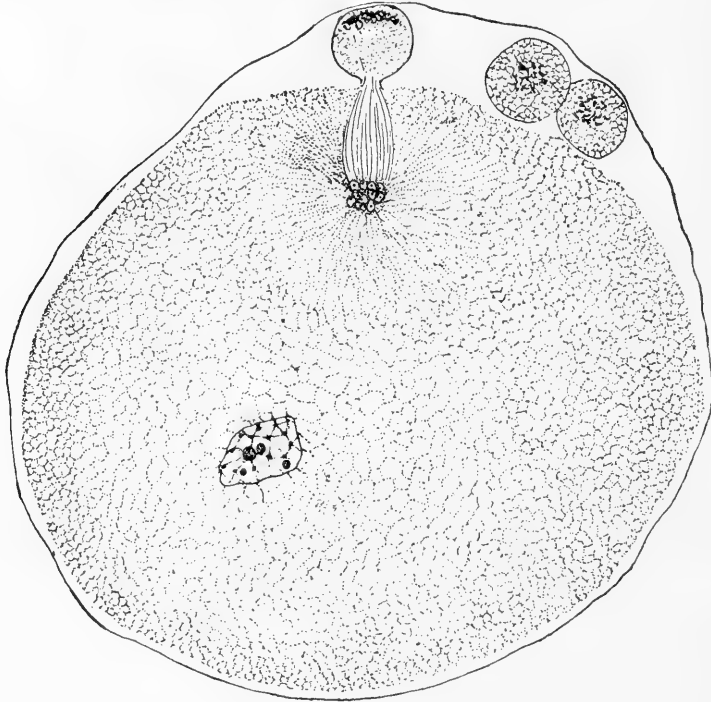


FIG. 6. Optical section of entire egg. First polar body divided. Second polar body in process of constriction. Vesicular chromosomes. Central aster of spindle. Early stage of male pronucleus.

cases some of the divisions contain no chromatin. Both polar bodies break up very soon into several spherical parts; and by the time the pronuclei are formed, there are frequently as many as ten spherical bodies of varying sizes distributed without any regularity, between the egg and its membrane (Fig. 7). Wilson (8) has already described this breaking up of the polar bodies.

The nucleoli, which are very pronounced in the germinal vesicle of the egg, prepared to leave the ovary, can be seen during the formation of the first maturation spindle, as bodies of varying sizes distributed through the cytoplasm of the egg. They appear first close to the spindle, and later some are seen very near the periphery of the egg (Fig. 1). These bodies were at first regarded by me as nucleoli; but later I was not satisfied with this interpretation, and I am greatly indebted to Dr. Wheeler for information which decides their significance. Upon this point he has generously given me the result of his unpublished work on the egg of *Myzostoma*.

After the first polar body has been constricted off, the eleven chromosomes remaining in the egg, move to the extreme lower pole of the spindle; and in one preparation they there assume the same form and arrangement that they show after the second polar body has been formed. In this case the entire achromatic portion of the second spindle must be a new formation. Fig. 6 shows the form and arrangement of the chromosomes after the second division, when the female pronucleus is entering upon the resting condition. Each chromosome has now assumed the form of a vesicle, with the chromatin occupying, in most part, the periphery of the vesicle, and examined from any point of view, they present the same appearance as indicated in the figure. The fact that exactly this same condition is seen after the formation of the first polar body, recalls Boveri's (3) statement that the claim has been made by Garnault and others that there is, in some cases, a resting stage between the first and second divisions. Whether this is a constant stage in the maturation of this egg, or an exceptional case, cannot be determined without the study of more material. This formation of chromatic vesicles, leading to a resting condition, offers a confirmation of the views of Van Beneden (1) in the case of *Ascaris*.

Pronuclei, Fig. 7. — The majority of eggs contain only two pronuclei, though apparently perfectly normal eggs may contain three or four. The size of the average egg at this stage of development is about .02 mm. smaller than the egg of the stage represented in Fig. 1. I have found no distin-

guishing characteristics of male or female pronuclei, either in size, shape or chemical reaction. Each contains the network, chromatin, and from one to seven nucleoli. The nucleoli persist during the cleavage spindle, but how much later, I am unable at present to state.

Polar rings (Figs. 7, 8).—Whitman (7) in 1878 published a detailed description of the dark areas at the two poles

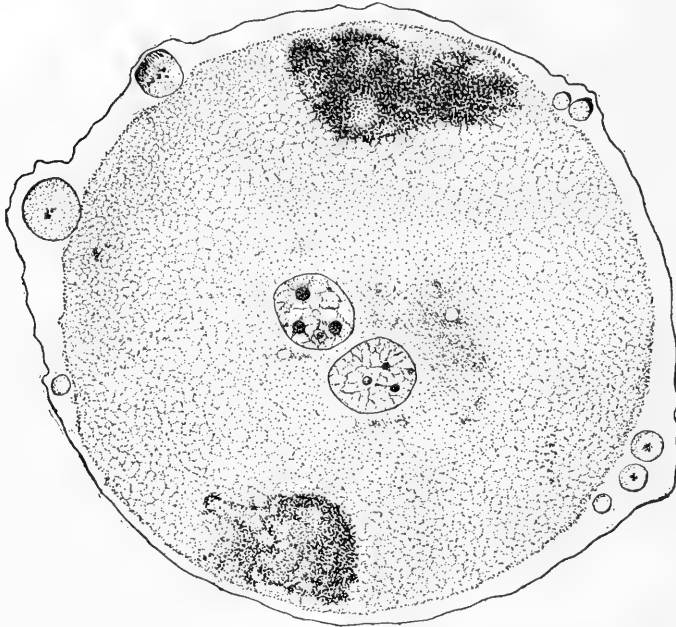


FIG. 7. Optical section of entire egg. Polar bodies divided into eight pieces. Polar-rings at opposite poles. Male and female pronuclei near center of egg.

of the egg of *Clepsine*, known as the polar rings. At that time he predicted that the "clear spot" described by Kowalevsky in *Rhynchelmis*, and interpreted as the germinal vesicle, would be found to be a polar ring. Vejdovsky (6) verified this prediction in 1892. I have found similar formations in the egg of *Allolobophora foetida*, appearing at opposite poles and attaining the maximum of their development at the time the pronuclei are formed. Figs. 7 and 8, show the form and size of the average rings. They are on the extreme periphery of

the egg, and have an appreciable depth of about 16 microns. The rings of the two poles differ in both form and dimension, and this seems to be a constant feature, making it possible to recognize one from the other. No two rings of either pole have been found exactly alike. The light areas in their center

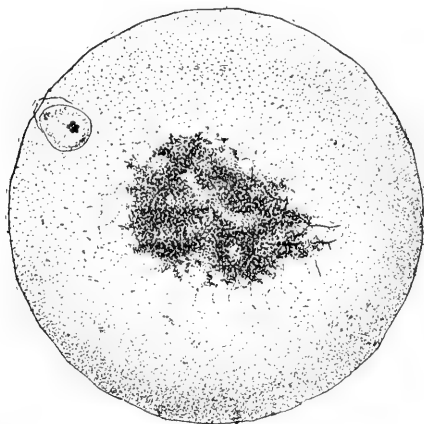


FIG. 8. Surface view of one polar ring. A fragment of polar body near the polar ring.

differ in size and shape, and the number and form of the smaller light areas, which occur through the mass of the rings, are by no means constant. The outer edges of the rings are never exactly alike in any two specimens. I hope in my future paper to be able to throw some light on the origin and destiny of these rings.

LIST OF PAPERS REFERRED TO.

1. E. VAN BENEDEN et A. NEYT. Nouvelles recherches sur la fécondation et la division mitotique chez l'*Ascaride megalocéphala*. *Bull. Ac. roy. de Belgique*, 1887.
2. TH. BOVERI. Zellen-Studien. Heft I. Jena, 1887.
3. *Id.* Heft III.
4. GARY N. CALKINS. On the History of the Archoplasm Mass in the Spermatogenesis of *Lumbricus*. *Trans. of the N. Y. Acad. of Sciences*. Vol. XIII.
5. R. FICK. Über die Reifung und Befruchtung des Axolotleies. *Zeitschr. für wissensch. Zoologie*. LVI. Bd., 4. Heft, 1893.
6. F. VEJDOVSKY. Entwicklungsgeschichtliche Untersuchungen. Prag, 1892.
7. C. O. WHITMAN. The Embryology of *Clepsine*. *Quart. Journ. Micr. Sci.* Vol. XVIII, 1878.
8. E. B. WILSON. The Embryology of the Earthworm. *JOURN. MORPH.* Vol. III, 1889.

THE SPHINCTER OF THE TERMINAL VESICLE OF HIRUDO MEDICINALIS.

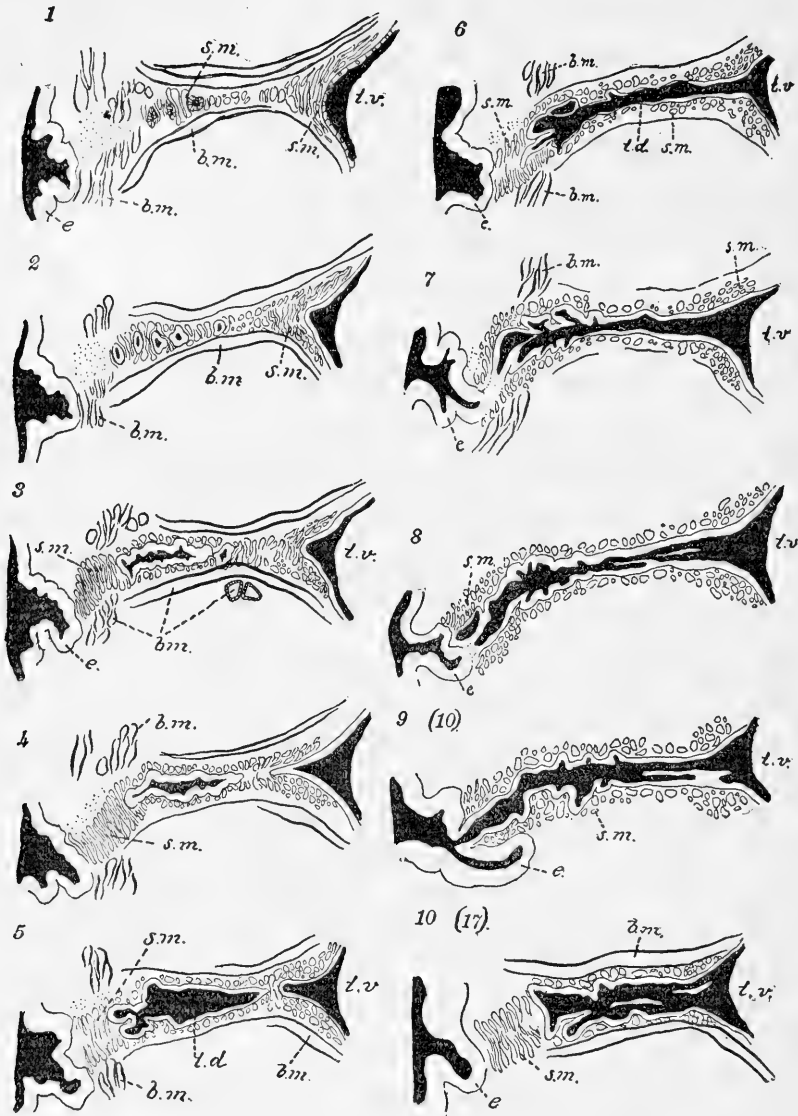
ARNOLD GRAF, PH.D.

IN a preliminary paper (*Annales de la Société Scientifique de Bruxelles*) H. Bolsius deals with my description of the "sphincter" in *Hirudo*, and promises to prove in a subsequent paper that I was wrong in my assertions.

I stated that the *sphincter*, discovered first by H. Bolsius, was not the simple structure that the drawings of this author would suggest, and gave a drawing of that organ in a paper on *Nephelis* (Beiträge zur Kenntniss der Excretionsorgane von *Nephelis vulgaris*. *Jenaische Zeitschr. für Naturw.* Vol. XXVIII, N. F. XXI). In this drawing (Pl. VIII, Fig. 8) I showed that instead of being constricted by one bundle of circular muscle-cells in one place only, *i.e.*, near the entrance to the terminal vesicle (as H. Bolsius figures it), the terminal duct is provided on its whole surface with circular muscle-cells. These cells increase in number in two places: first, near the communication of the terminal duct with the terminal vesicle; second, near the aperture of the terminal duct to the exterior. I treated this fact in my paper as of no great consequence, not being directly related to the facts interesting me at that time. However, H. Bolsius seems to consider it as of great consequence, and asserts that my observation was wrong, suggesting that I took the "*body muscle-cells*" for sphincter muscle-cells.

When H. Bolsius informed me by letter that he had written a paper on this subject, I looked over my series, almost inclined to think that I was really mistaken. But to my great satisfaction I found that I was right; and I try to prove this by the figures in this paper.

The figures represent successive longitudinal sections through the terminal duct, with the sphincter muscle-cells, and are drawn with the camera of Zeiss under a magnification of 112.



Figs. 1 to 8 are successive sections. Between Figs. 8 and 9 (10) one section is omitted, which shows nothing of importance. In Section 10 (Fig. 9 [10]) half of the terminal duct is cut through, and this should be sufficient for the proof of my assertions. Nevertheless, I have added one figure (Fig. 10 [17])

representing the seventeenth section of the series. The lumen of the terminal duct (*t.d.*), part of the terminal vesicle (*t.v.*), and the exterior are filled out with black ink in the drawings.

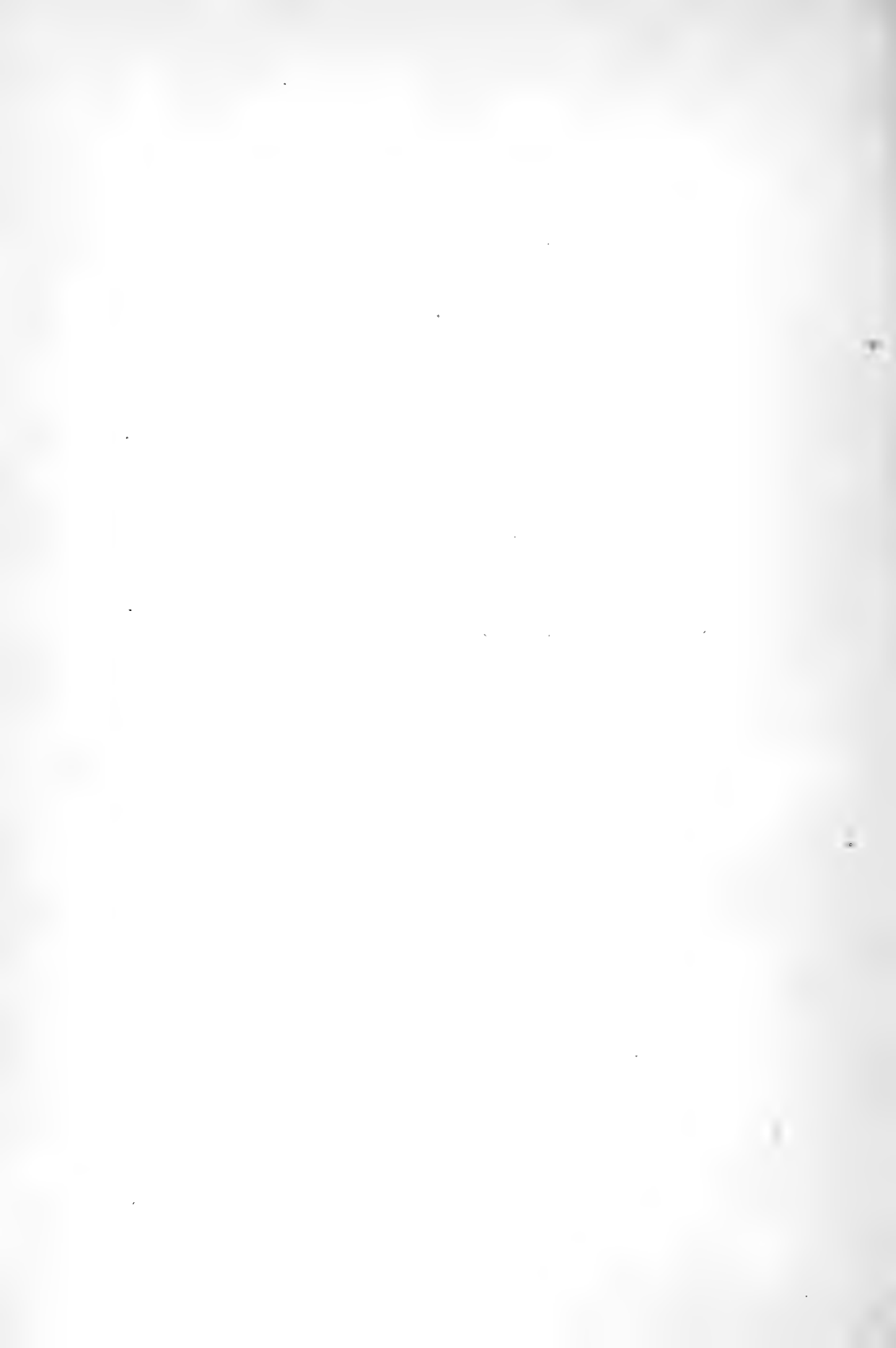
In Figs. 1 and 2 the terminal duct is cut only in a few places. In the middle and close to the terminal vesicle we see longitudinal sections through circular muscle-cells (*s.m.*) as we have to expect in a surface section. Figs. 3 and 4 show part of the lumen of the terminal duct in the middle (black), and now we meet longitudinal sections through the sphincter muscle-cells, close to the epidermis (*e*). In the places where (in Figs. 1 and 2) we saw longitudinal sections through sphincter muscle-cells, we find here cross sections. In Figs. 5 and 6 the sections of sphincter muscle-cells close to the epidermis shorten up, until, in Figs. 7, 8, and 9, we find all the sphincter muscle-cells in cross sections at the two sides of the terminal duct now showing its lumen entirely.

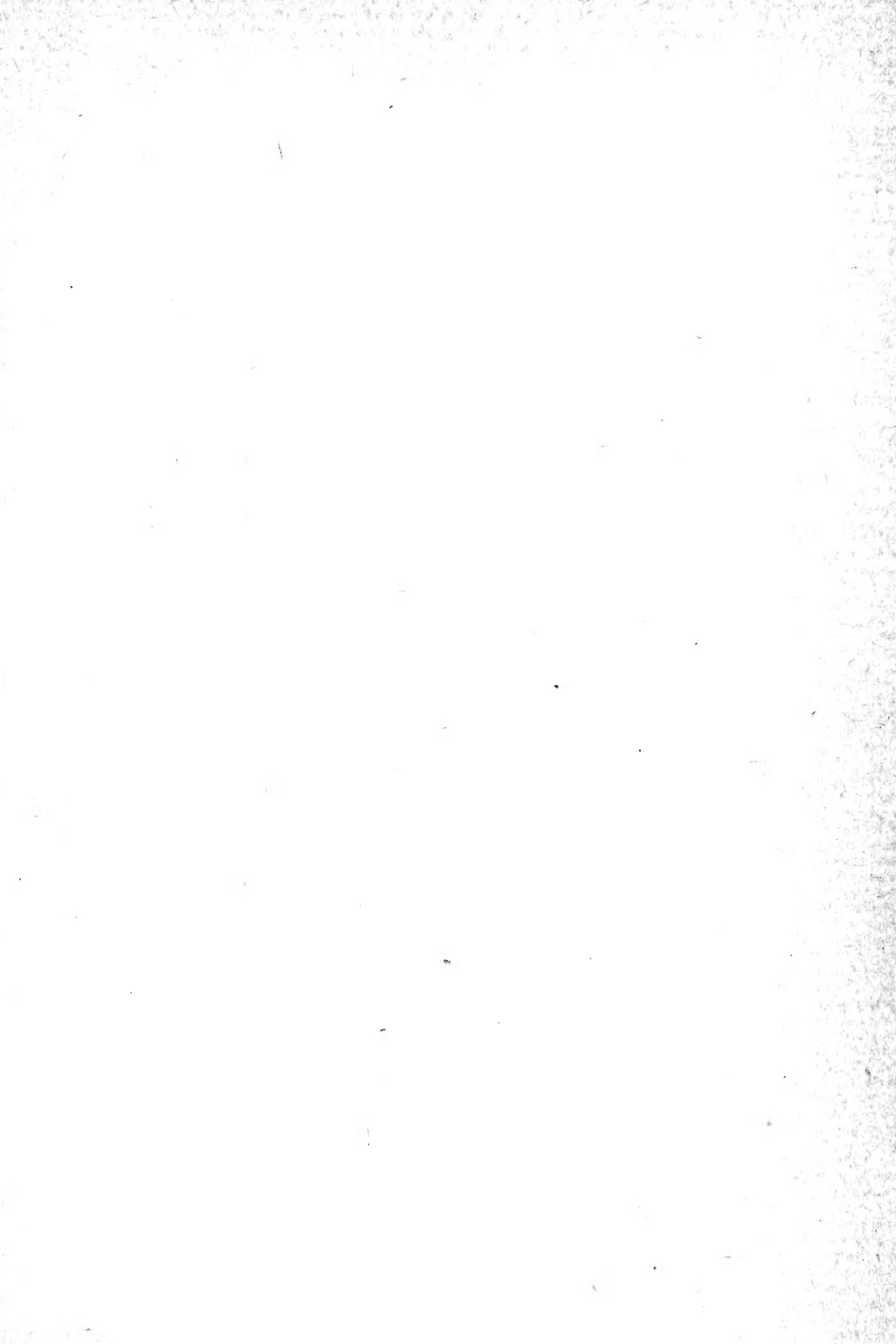
In the following sections the same pictures are repeated, only in reversed way. Fig. 10 (17) gives a view of section 17 of the series which would almost exactly correspond to Fig. 3.

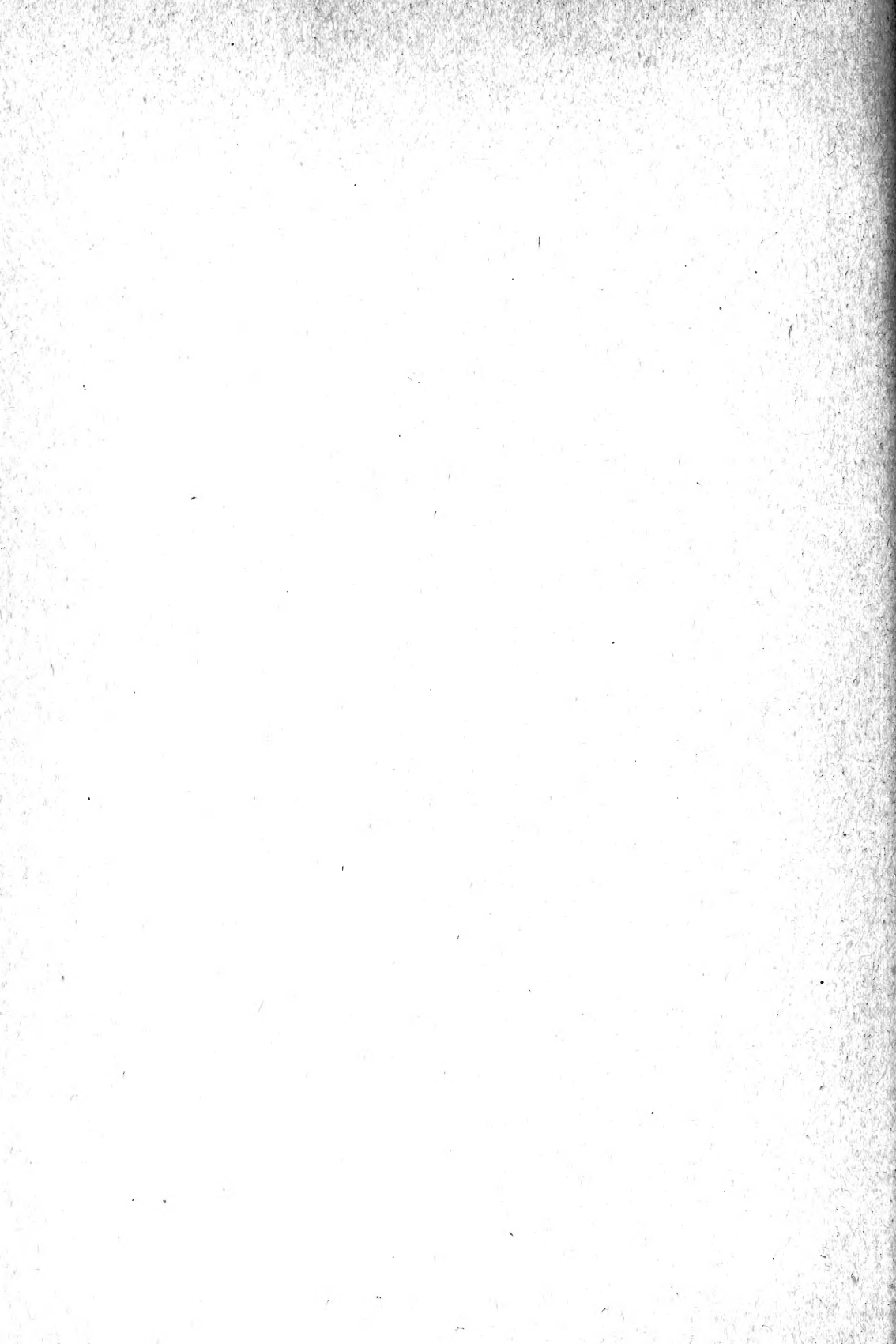
If H. Bolsius suggests that I mistook body muscle-cells for sphincter muscle-cells he must take me for a very superficial observer. The difference in size and structure is so great between those two kinds of muscle-cells that nobody could mistake one for the other. The body muscle-cells are, on the one hand, four times as large as the sphincter muscle-cells; and, on the other hand, the body muscle-cells are striped longitudinally and show the fibrils deeply stained; whereas the sphincter muscle-cells are smooth and are much less stained.

MARINE BIOLOGICAL LABORATORY, WOOD'S HOLL,

August 9, 1894.







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